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PLANT PHYSIOLOGY

(FIZIOLOGIYA RASTENII)

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PATHS AND MECHANISM OF MINERAL SUBSTANCE MOVEMENT FROM ROOTS TO ABOVE-GROUND PLANT ORGANS AS EXEMPLIFIED BY P^{32} TRANSPORT

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Questions of movement of mineral substances in plants have become the chosen province of phytophysiology, in which radioactive isotopes were first applied experimentally.

Among the new facts established by this method, special interest was aroused by the possibility of rapid lateral (side-wise) movement of mineral substances from the woody tissue to the cortex [1]. Based on these data, some investigators [2, 3] are inclined to consider it as finally proven at this time that the channel of the ascending flow of mineral substances in the plant is the xylem, while the tendency is to ascribe transmission of labeled mineral substances in the plant phloem to a fractional transition of mineral substances moving along the woody tissue into the adjacent sectors of the cortex.

It should be mentioned that this conclusion was made despite the fact that the first experiments employing radioactive isotopes (P^{32}) afforded a basis for a completely opposite conclusion, namely, that the basic path for the ascending current of mineral substances in the plant is the phloem or, at any rate, the phloem equally with the xylem [4].

We believe that these contradictions did not all happen by chance. They reflect the complexity of the phenomenon in itself, its dependence on a number of factors which determine the relative participation of active and passive links in the general absorption process, as well as movement of mineral substances in the plant.

In due course we succeeded in showing [5], as exemplified by the absorption and transmission of potassium, that the effect of the transpirational current intensity on the absorption and transport of mineral substances in the plant is not and cannot be well-defined. Depending on the physiological state of the plant, particularly on the saturation level of its tissues by mineral substances, this effect may in some cases be totally absent (at a very low level of tissue saturation by mineral substances), while in other cases (at a high saturation level) it may have the effect of a decisive factor. We saw the explanation of this circumstance in the fact that at low saturation of tissues by mineral substances, the latter are transported chiefly by the living elements of phloem on the order of surface migration of ions, i.e. independently of water movement. Transport of ions in this case is determined by metabolic factors, primarily by the respiratory process of living conducting tissues.

As the degree of saturation of living tissues by mineral substances is increased, the path of active movement along the phloem gradually overlaps or becomes more difficult, and under these circumstances a greater role is acquired by s desorption of mineral substances into xylem tissues and their subsequent passive transport with the liquids of tne transpirational current.

In these investigations we conducted no direct observations on transport of potassium,and therefore, it was interesting to clarify the question of cortex and woody tissue participation in the transport of mineral substances by using labeled atoms for the purpose. Considering the short life of radioactive potassium (K^{42}), we thought it more advisable to use for these purposes labeled phosphorus (P^{32}), which moves easily through the plant.

TABLE 1

Ash Content in Cotton Plants Under Investigation at the End of the Preparatory Period (on dry basis)

Experiment variant	Leaves	Cortex	Woody tissue	Roots
Plants were held for 9 days before testing:				
On tap water	16.7	7.4	3.5	6.3
On a complete nutrient solution	19.5	8.9	5.1	8.8

TABLE 2

Effect of Prior Nutrient Conditions on P^{32} Distribution Between the Cortex and Woody Tissues 1 Hour and 4 Hours After Placing Plants in a Solution Containing P^{32} (pulses per 100 mg of dry substance per minute)

Experimental variants	Stem cuttings under analysis						Ratio of cortex : woody tissue in analyzed stem cuttings		
	lower		medial		upper		lower	medial	upper
	cortex	woody tissue	cortex	woody tissue	cortex	woody tissue			
Plants held for 9 days before the experiment:	After 1 hour								
On tap water	124	116	170	131	373	235	1.07	1.30	1.59
On a full Hellriegel nutrient solution	150	395	181	571	218	692	0.38	0.31	0.32
Plants held for 10 days before the experiment:	After 4 hours								
On Knop nutrient solution diluted 1:4	6242	10702	8638	14290	9940	20757	0.58	0.61	0.48
On Knop nutrient mixture double strength	743	1568	1223	2289	2567	6497	0.47	0.53	0.40

Based on the concept mentioned above of a difference in participation of the cortex and woody tissue in movement of mineral substances, depending on the degree of plant saturation by these substances, we considered it necessary to investigate primarily the distribution of P^{32} moving along the plant between the cortex and woody tissue, in relation to the indicated difference in the conditions of the test plants.

The experiments were conducted with cotton plants, variety 108F. The plant preparation for experimentation was processed as follows: the plants were grown for 51 days in aqueous cultures on a diluted Hellriegel mixture (phosphorus content $^1/_4$ of the customary Hellriegel concentration and all other elements at a concentration $^1/_2$ the usual one).

Subsequently the plants were divided into 2 groups, one of which was placed for 9 days in a fresh Hellriegel solution of normal concentration, and other other group for the same period in tap water (without addition of nutrient mixture). In each group there were 12 vessels, each containing one plant. During the 9 days of the preparatory period, differences were created in the degree of saturation of mineral substances. It can be seen that this aim was accomplished from analysis of individual organs and portions of investigated plants for ash content at the time of their transfer to a solution with P^{32} (Table 1).

At the end of the preparatory period all plants under investigation were placed on tap water to which sodium phosphate containing P^{32} was added, on the basis of 50 microcuries per vessel of 1 (liter) capacity. The solution reaction was adjusted to pH 5.2. Exposure for 1 hour.

After one hour's interaction of the roots with the solution containing labeled phosphorus, the above-ground plant mass was cut down, and for purposes of radiometric analysis for P^{32} content in the cortex and woody tissue three cuttings of the stem were taken, beginning with a height of 6 cm from the base. The length of each cutting was 4 cm. After separating the cortex from the woody tissues, the samples were dried, ground, and radioactivity measured with a Geiger-Müller counter (B-2 counter).

Results are given in Table 2, from which it can be seen that in the case of plants weakly saturated by mineral substances, the radioactive phosphorus transported along the plant is concentrated in the cortex to a greater degree than in the woody tissues (the ratio of radioactivity of the cortex to woody tissue is greater than unity). In the case, however, of plants with greater saturation by mineral substances, the main flow of P^{32} is found in woody tissues (the ratio of radioactivity of the cortex to woody tissues is considerably less than unity).

This was the case in several starved plants upon intermittent exposure, in which the intake of P^{32} into the tissues was still not large.

When the exposure period is prolonged and the content of P^{32} in the tissues is increased as a consequence, the indicated differences in distribution of P^{32} between the cortex and woody tissues in plant differently prepared for the experiment becomes less marked, although even here, in a number of cases, they are quite marked. Thus, in one of our experiments in which the exposure of plants to a solution with P^{32} continued for 4 hours in environments of lower air humidity (a humid chamber) the following radioactivity indices were obtained for the cortex and for woody tissues in different sectors of the stem, as shown in the lower portion of Table 2.

In this experiment the preparation of plants weakly saturated by mineral substances was conducted by placing them not on tap water, but on a Knop nutrient mixture diluted four-fold. These plants, unlike the severely starved plants in the preceding experiment, absorbed P^{32} with considerably greater intensity than the highly saturated plants, which were placed in double strength Knop solution.

Even under these conditions the weakly saturated plants had a noticeably broader ratio of cortex and woody tissue radioactivity than highly saturated plants, i.e. even here the P^{32} flow passed principally along the cortex.

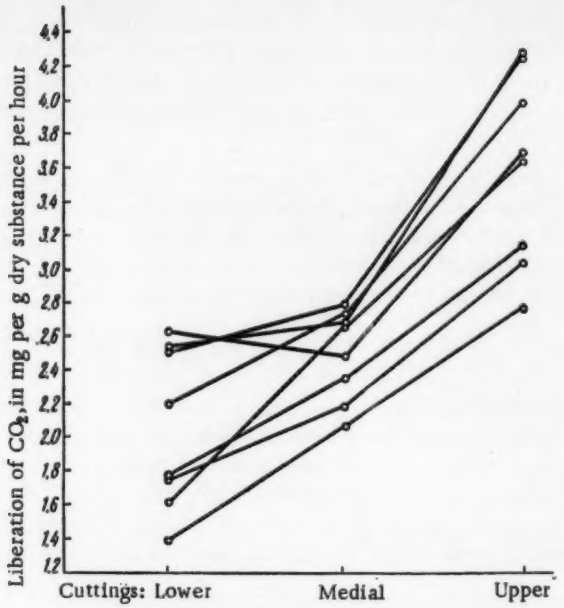
In Table 2 the clearly expressed gradient of P^{32} concentration in the stem is conspicuous, directed from the top downward. This phenomenon was repeated many times also in a number of other experiments. It proved to be closely related to the gradient of respiration intensity of the cortex in stems of experimental plants.

Thus, in the figure, results are given of the respiration intensity calculated by the Boysen-Jensen method, modified by S. V. Tageeva, in cortex cuttings along the stem, taken at different times in a number of differently prepared plants, before they were placed in a solution with P^{32}.

As can be seen from the Figure, the gradient of respiration intensity in the cortex in almost all cases shows a clearly expressed direction from the top downward, as well as a concentration gradient of P^{32}. It must be assumed that this intensity gradient of cortex respiration determines the active movement of P^{32} along the cortex from below upward against the concentration gradient.

In relation to this there should be recalled the studies of Kursanov and Turkina [6], who showed on isolated vascular-fibruous tufts of sugar beets and plantain, as well as on bast fiber cuttings from young sprouts of yellow acacia, an accelerated respiration on contact of one end with a solution containing a substance capable of transmission along the plant (in the given case – sucrose). Our data, which establish a relationship between the intensity gradient of cortex respiration along the stem and P^{32} movement, can also be interpreted as an argument in favor of an active nature in the process of movement of substances in the plant. However, this transport mechanism, as we have seen, is found to have a definite relationship to the simultaneous passive transmission of these substances with the fluids of transpirational flow along the dead xylem vessels.

What are these relationships? The answer to this question requires careful experimental analysis. The point is that, based on the studies mentioned at the beginning of this article which revealed the possibility of a speedy lateral movement of mineral substances from woody tissue to the cortex, all the facts stated by us on distribution of P^{32} between the cortex and woody tissue could be explained without admitting participation of the cortex as a channel for ascending currents of substances in the plant. It is sufficient to acknowledge that in all cases, the radioactive phosphorus found in the cortex was absorbed from woody tissues as the ascending flow



Respiration intensity of cotton plant cortex taken at different portions of the stem.

TABLE 3

Effect of Stem Ringing on the Intensity of P^{32} Intake in the Cortex and Woody Tissue (pulses per 100 mg dry substance per minute)

Point at which the stem cutting was taken	Nonringed plants		Ringed plants	
	cortex	woody tissue	cortex	woody tissue
Below the ring	1277	2542	1891	4588
Region of the ring	1611	2586	—	27380
Above the ring	2011	3385	3865	13586

of P^{32} passed through, and that the intensity of this process depends on the degree of cortex saturation by mineral substances, the respiration intensity of different cortex portions, etc. With such an explanation there could be assurance in recognizing that the woody tissue is the only channel of ascending flow for mineral substances in the plant.

The reason for rejecting this viewpoint as the only one is based on our observations of transport characteristics in transport of P^{32} by plants whose stems were ringed. These observations were conducted on plants with a moderate mutrient background.

The interaction of roots with the solution containing P^{32} lasted 6 hours. Before being placed in this solution a part of the plants was ringed at a height of 6 cm from the stem base. The width of the ring — 2 cm. After a 6-hour exposure in the P^{32} solution, cuttings of the stem above and below the ring, as well as of the woody tissue of ringed portion, were taken for calculating radioactivity. The same cuttings at the same heights from the stem base were also taken for the control (nonringed) plants. The results of radioactivity calculations of the cortex and woody tissue from the stem cuttings taken are reported in Table 3.

A similar result was obtained also in another experiment with plants on a lower nutrient background.

The data in Table 3 disclose new mechanisms which, in our opinion, are of great significance in principle for understanding the problem under investigation.

It can be seen from Table 3 that in the control (nonringed) plants, the region of the ring gives indices of woody tissue radioactivity closely related to indices of woody tissue of the stem-cutting below, and somewhat less than the indices of woody tissue above the cutting. An entirely different picture is shown by the ringed

plants. Here the woody tissue radioactivity in the region of the ring exceeds by several times the woody tissue radioactivity of stem cuttings below as well as above the ring. The impression is created that while the ascending P^{32} flow did not reach the ring, it was distributed in one or another relationship between the cortex and woody tissue, moving in a wide channel represented by these two conducting paths.

When it strikes a ringed section, the P^{32} flow, directed by the single remaining stream (that of woody tissue) increased markedly, like that of a stream of water rushing through a narrow gorge. As shown by Table 3, in this case the woody tissue radioactivity of the ringed section is increased more than 6 times by comparison with the woody tissue radioactivity of the section below. After passing through the narrow passage (the ringed section), the P^{32} current again flows through a wide channel which embraces the cortex and woody tissue, and is expressed by diminishing the radioactivity index of the woody tissue by one-half.

From these data an important deduction is arrived at: the movement of P^{32} from the cortex into the woody tissue (when the P^{32} flow reaches the ringed section) occurs just as easily as the movement from the woody tissue into the cortex, commonly acknowledged at present (in our case, after passing the ringed section). In other words, the ascending P^{32} current flows along the plant in constant energy interchange between the cortex and woody tissue, concentrating, depending on conditions, chiefly in the cortex in one case, and in another, chiefly (when the whole cortex is removed) in the woody tissue.

With respect to the data outlined, it might be suspected that a marked jump in woody tissue radioactivity in the ringed sector is due to increased evaporation of water from this denuded portion of the woody tissue, deprived of cortex. Together with the evaporated water there might have been an increased inflow of P^{32} into this sector. Even though measures were taken in conducting the stated experiment to eliminate excessive water evaporation from the denuded woody tissue surface (the ringed sector was carefully wrapped in parchment paper), the experiment was subsequently repeated under conditions of air humidity close to total saturation (nearly 100% as determined by an Assman psychrometer) so as to eliminate this objection completely. For the duration of the experiment on P^{32} absorption, the plants were placed in a roomy glass-enclosed chamber in which the high air humidity mentioned was created and retained during the whole 6-hour experimental period. The details of preparing the plants and conducting the experiment were the same as in the experiment described above. Plants weakly saturated by mineral substances were used in this experiment. The results are reported in Table 4.

The data of Table 4 are a repetition of the effect familiar to us found in atmospheric conditions saturated by humidity. The data of this experiment are the more convincing in that they, unlike the data of the previous experiment, do not produce an increase of the general P^{32} flow in the ringed plants; this flow increased markedly only in the ringed portion of the woody tissue.

It should be noted that the very fact of unhampered P^{32} intake into plants at such high air humidity, which leads to severe inhibition of transpiration, militates against the assumption of transpiration playing the decisive role as the only stimulant of the ascending flow of substances in the plant, and of the woody tissue as the only channel of this flow.

An important argument against acknowledging the essential role of the cortex in the ascending flow of substances is found in the results of experiments showing the effect of detaching the cortex on the transport of labeled mineral substances. Actually these experiments, which showed that the content of labeled mineral substances (phosphorus, potassium, sodium) in the detached cortex is quite insignificant by comparison with their content in sections below and above locations with undetached cortex, as well as by comparison with their content in woody tissues of the detached section — these experiments served as a basis for conclusions on transport of substances along woody tissues and the accompanying lateral movement from woody tissue into the cortex.

Our experiments on detaching the cortex, confirming the fact of difficulty in transmitting P^{32} along the detached section, developed along with this the possibility of another interpretation.

The experiments were conducted with the same cotton plants, prepared for the experiment by the method described above. The plants were placed in tap water for the 9 days of the preparatory period. The separation of the cortex from woody tissue was made in the middle portion of the stem for a distance of 4 cm. After a circular detachment of the cortex from the woody tissue, a strip of paraffined parchment paper was drawn through an incision in the cortex, isolating the cortex from the woody tissue. Subsequently this section of the stem was

TABLE 4

Effect of Stem Ringing on the Intensity of P^{32} Intake Into the Cortex and Woody Tissue Under High Air Humidity Conditions (pulses per 100 mg dry substance per minute)

Points at which the stem cutting was taken	Nonringed plants		Ringed plants	
	cortex	woody tissue	cortex	woody tissue
Below the ring	4172	6541	2290	6674
Region of the ring	4374	7055	—	16185
Above the ring	—	7782	2857	8282

TABLE 5

Effect of Cortex Removal on the Intensity of P^{32} Intake Into the Cortex and Woody Tissues (pulses per 100 mg of dry substance per minute)

Origin of stem cutting	Control plants		Plants with cortex removal	
	cortex	woody tissue	cortex	woody tissue
Below the zone with cortex removal	733	1121	1564	2916
Zone with removed cortex	739	1251	82	6188
Above the zone with cortex removal	1255	2165	1816	4869

carefully wrapped in paraffined paper on the outside to prevent evaporation. Exposure to a solution containing P^{32} continued for 6 hours under normal conditions of transpiration (a cloudless day).

Results of radioactivity measurement in different sections of cortex and woody tissue in experimental plants and controls (without detaching of cortex) are reported in Table 5. The average cutting corresponds to that portion of the stem where in experimental plants the cortex was detached, while it was left whole in the control.

From Table 5 it is evident that in the detached cortex the radioactivity is markedly lowered. At the same time, a marked rise of radioactivity is observed in the adjacent section of woody tissue. In other words, detaching the cortex brought on the same effect as the ringing did in experiments described above. It is important to emphasize that in the detached cortex some, even though a much lower, radioactivity is still noticeable, which indicates an ability in principle of transporting P^{32} upward along the cortex. However, detachment of the cortex severely limits this ability. The explanation of this fact may be seen in that the ascending current of mineral substances flows along the stem as a complete system with a constant exchange between the cortex and woody tissue. The detaching of the cortex destroys this completeness, which leads to the shifting of P^{32} flow chiefly to woody tissue. The high saturation of the cortex living elements by mineral substances also leads to the same phenomena which were shown above.

That the entire ascending P^{32} flow can be shifted experimentally also onto the cortex can be concluded from experiments by Gustafson and Darken [4] which showed that on removal of a small section of woody tissue, the radioactivity of the cortex and the woody tissue of the stem cuttings located above it, into which P^{32} could penetrate only by movement through the cortex, differed but little from the radioactivity of corresponding cortex and woody tissue cuttings of plants with undisrupted woody tissue. *

Our subsequent investigations have shown that even in the case of cortex removal and the preservation of the integrity of woody tissue, considerably greater P^{32} movement along the split-off cortex can be attained than in the experiment just mentioned in which the magnitude of radioactivity of the removed cortex exceeded the

* In a subsequent article by Gustafson [7], no significant movement of P^{32} along the cortex was found. However, in this work, by contrast to previous ones, the statement is not of a direct determination of P^{32} content in the cortex and woody tissue of the stem above the zone of interrupted transport along the woody tissue, but of measurement of the intensity of general accumulation of P^{32} in the entire above-ground mass above this zone for a comparatively long period of observation (up to 69 hours). It is quite evident that these data decide not the problem of the principle of possible participation of the cortex as a channel of the ascending flow of mineral substances in plants, but the problem of the effect of a cutting operation on woody tissues on P^{32} accumulation in the plants. If it is taken into account that in these experiments, in cases of cutting the woody tissues, one cannot successfully avoid a considerable wilting of leaves despite any measures to prevent it, then a very weak accumulation of P^{32}, found by the author in plants with cut woody tissues, is by no means unexpected.

background readings only four-fold (19-20 pulses per minute). It is also most important that in these investigations we succeeded in attaining an arbitrary change of magnitude of radioactivity found in the removed cortex by changing the intensity of the general P^{32} flow along the plant.

In one of the experiments with cotton plants, grown on $^1/_2$ strength Knop mixture, 3 groups of plants were created with different capacities for absorbing and transporting P^{32}: 1) plants placed for 10 days before the experiment of P^{32} absorption on double-strength Knop mixture; 2) plants placed for the same time on $^1/_4$ strength Knop mixture; and 3) the same plants as in the second group but treated twice after the cortex was removed, before being placed in a P^{32} solution, by a solution of 2, 4, 5-trichiorophenoxyacetic acid (TA) in a concentration of 50 mg per liter. The latter was done to increase the general P^{32} flow directed from the roots into leaves and uppermost points of growth.

After a 4-hour exposure to a solution with P^{32} under conditions of lowered transpiration (humid chamber), the following levels of radioactivity were found in the cortex of control plants (without cortex removal) and experimental plants (Table 6).

As seen from Table 6, the consecutive increase of general P^{32} flow along the plant from the first to the third group is accompanied also by a marked increase of P^{32} content in the removed cortex, even though it is less than the P^{32} content in the unremoved cortex of control plants in the same zone of the stem, as well as in the cortex in the stem sections of experimental plants above and below the zone of removal.

TABLE 6

Effect of Cortex Removal on Its Intake of P^{32} at Different Intensities of P^{32} Flow Along the Plant (pulses per 100 mg of dry substance per minute)

Cutting taken from	Highly saturated plants		Weakly saturated plants		
	control plants	plants with detached cortex	control plants	plants with detached cortex	plants with detached cortex treated by TA
Below the zone of removal	743	1656	6242	6724	10263
Zone of removal	1223	463	8630	1652	1987
Above the zone of removal	2567	2941	9940	12868	18167

Taking into account the high absolute indices of radioactivity in this experiment, which even in the detached cortex exceed by many tens of times the background index (19-20 pulses per minute), the data reported in Table 6 can be accepted as convincing proof in principle of the possibility of P^{32} transport also along the cortex detached from the woody tissue.

It is of interest that in these conditions of intense P^{32} transport even along the detached cortex, there was a decreased need for such marked channeling of the P^{32} flow into the woody tissue of the zone of removal as that observed in the experiment reported in Table 5, in which conditions for P^{32} transport along the cortex were hardly favorable. Actually, the determination of woody tissue radioactivity in weakly saturated plants in the experiment just reported showed that in the transport from the lower (integral) stem zone into the medial (with detached cortex), the woody tissue radioactivity is increased 1.5-1.6 times, instead of 1.3 times in the control plants, while in the experiment reported in Table 5, the radioactivity of woody tissue in the detached zone of experimental plants increased by 2.1 times, instead of 1.1 times in the control plants. These figures confirm the conclusions above that the marked increase in woody tissue radioactivity in the section with detached cortex is due to transport of P^{32} from the cortex into woody tissue when the P^{32} flow reaches the section with detached cortex. In other words, these data lend support to the theory not only that the movement of P^{32} along the cortex is perfectly feasible, but also that the P^{32} transport from the cortex into woody tissue is accomplished as easily as its transport from the woody tissue into the cortex.

The circumstance that Stout and Hoagland [1] could detect a noticeable radioactivity in the cortex split off from woody tissue must to a considerable degree be explained by the fact that they dealt with plants cultivated on a rather high nutrient level (the nutrient mixture they used exceeded by several times in concentration of most mineral substances that of Hellriegel mixture), while Gustafson and Darken [4] dealt with plants very weakly saturated by mineral substances (cuttings of Sedum and Bryophyllum were placed in sand, and subsequently transferred to vessels with tap water).

Further, in experiments of Stout and Hoagland the transpiration of experimental plants was heightened by use of a fen, which in plants highly saturated by mineral substances increased the transport of substance even faster, particularly along woody tissue by-passing the detached section of cortex.

All the foregoing leads to the conclusion that the movement of mineral substances (in any case, many of them) upward along the cortex is fully possible; that in the plant, on the whole, this movement is realized at a constant exchange of mineral substances between the cortex and woody tissues in the adjoining sections, and when the plant experiences a marked deficit in nutrient substances there is always a path open for their transport from roots into above-ground organs, activated by the life activity of living conducting tissues of the plant, and one totally independent of such an unstable and unreliable factor as the transpirational flow.

If one considers that existence in plants of a downward flow of substances, in any case of plastic substances, along the cortex is no longer to be doubted, then from all that has been said, the conclusion also follows that the cortex may serve simultaneously as a channel of upward and downward flow of substances. This position is strengthened by the experiments of Chen [8], who observed movement of P^{32} and C^{14} along geranium cortex in apposite directions. The possibility not only of a downward but also of an upward flow of substances in the phloem, i.e. independently of movement of water, was shown in investigations of Kursanov and Zaprometov [9] on the basis of amino acid movement to ripening grain seeds. Finally, we should note that in our investigations it was shown [10], in turn, that in interaction of barley roots with a suspension of soil richly saturated by sodium, the plant produces two cation currents in opposite directions which penetrate the whole plant – a current of sodium ions in the ascending flow and desorption from the plant of calcium, potassium, and magnesium ions in a descending direction. It can be assumed that in the given case these two counter cation currents occur in the phloem.

SUMMARY

1. As a further development of a suggestion made by one of the authors it is shown by experiments with P^{32} that the distribution of P^{32} moving along the cortex and wood intimately depends on the degree of saturation of the plants with mineral substances. In highly saturated plants largest amounts of P^{32} are found in the wood, and in weakly saturated plants – in the cortex.

2. The concentration gradient of P^{32} in the stem (from top to bottom) coincides with the direction of the respiration gradient in the same cortex sections of the stem. This circumstance may explain the movement of P^{32} along the cortex against the concentration gradient.

3. Experiments on ringing or separation of the bark give reason to believe that translocation of mineral substances (P^{32}) in plants as a whole occurs during a continuous exchange of these substances between the cortex and wood in the adjacent layers.

4. Violation of wholeness of the stem by separation of the bark sharply decreases the movement of P^{32} along the cortex and results in the P^{32} current moving mainly along the wood in the denuded sections. However in weakly and moderately saturated plants under conditions of weak transpiration, P^{32} is detected in the detached cortex in such amounts which leave no doubt that in principle P^{32} can also move along the detached cortex.

5. The sum total of the data permit one to conclude that in plants deficient in nutrients, translocation of the latter from the roots to aerial organs is independent of movement of water. It can take place in living plant tissues, being activized by the life activity of the latter.

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NITROGENOUS SUBSTANCES IN THE SAP OF PUMPKIN

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Sabinin [1], Shmuk and students [2, 3], Kursanov and colleagues [4] and Mothes and colleagues [5] have demonstrated that the root system of plants must have both the apparatus which serves in the absorption of food from the soil, and those which serve the growing organism where synthesis of various organic compounds takes place.

One of the methods used in the study of the synthesizing activity of the root system is the analysis of sap which is found under various conditions of plant foods. It was obvious that this method could give valuable results in the study of the biochemical processes which are the basis for the assimilation of the inorganic forms of nitrogen and the transformation of it into organic nitrogen compounds through various types of synthesis in the plant.

Bykov [6] showed that a portion of the inorganic nitrogen located in the root system is held in the tissue of the root and there undergoes transformation. Litvinov [7], in studying the sap of pumpkin, showed that a significant amount of nitrates and organic acids, and a number of amino nitrogen compounds were found in the sap. Using paper chromatography many investigators [8-12] discovered amino acids in the sap of row plants.

There are now experimental data which show positively that the process of converting the inorganic nitrogen coming in from the soil into organic nitrogenous substances takes place in the root system. However there are still insufficient quantitative data for the determination of the composition of the nitrogenous parts of the sap under different conditions of nitrogen food levels. The question of the presence of protein in the sap also arises: protein substances, which coagulated on heating, were shown in connection with the sap only in the work of Litvinov [8].

The problem undertaken in this work was the study of the composition of the nitrogenous substances in the sap of pumpkin fertilized with nitrate and ammonium fertilizers in cultivated soil.

The work was carried out with the sap of Gribovskii 31 pumpkin hybrid. The pumpkins were grown in boxes on a light sandy loam mixed with peat. A normal amount of fertilizer was combined according to the direction of Gedroitz [13].

There were 15 kg of soil in every box. In the experiment with ammonium fertilizer, the following food mixture was placed in the box prior to planting: $(NH_4)_2SO_4$ – 13.1 gr (figures at 0.5 gr, nitrogen to 5 kg soil); $NaH_2PO_4 \cdot 2H_2O$ – 3.0 gr (figures at 0.5 gr P_2O_5 to 5 kg soil); K_2SO_4 – 1.11 gr (figures at 0.2 gr K_2O to 5 kg soil).

For a measure of plant growth, a half strength application of $(NH_4)_2SO_4$ in a mixture of 0.25 gr nitrogen to 5 kg soil was also used.

In the other half of the boxes nitrogen was applied in the form $Ca(NO_3)_2$. The following were applied prior to planting: 12 gr $Ca(NO_3)_2 \cdot 4H_2O$ (at the rate of 0.5 gr nitrogen to 5 kg soil); $NaH_2PO_4 \cdot 2H_2O$ and K_2SO_4 applied at the same rate as in the first case. A half strength mixture of $Ca(NO_3)_2 \cdot 4H_2O$ was applied in such amounts that there was 0.25 gr nitrogen for each 5 kg soil, in order to have a measure of plant development.

The pumpkin was grown for 40-50 days. During the summer and fall the plants were grown in a vegetable shed, and during the winter and spring under greenhouse conditions with artificial light. Collection of sap was carried out in the following manner. A strip of filter paper was placed around the stem of the plant to protect the stem and sap from soil particles, and the stem was carefully rubbed with alcohol; after this, the stem was cut with a razor at a height of 2-3 cm above the root collar, rubbed with alcohol, and a sterilized rubber baby bottle was placed at the narrow end of the cut, where a small opening had been made earlier. The wide opening of the bottle was closed with a sterile wad. When sap had accumulated, generally after 2-3 hours, it was collected with a sterile pipette and put into sterile jars with a ground-in-stopper, and placed under refrigeration at 0°. Sap collection continued for one day. The sap from plants which had been fertilized with ammonium salts was labeled "ammonium", and that from the plants fertilized with nitrates was labeled "nitrate". In all cases the dry weight, ash content, and pH of the sap were also determined.

The study continued during the summer, winter and spring of 1956-57. The summer plants were larger and gave significantly greater quantities of sap than the plants grown during the fall and winter, in spite of the exact humidity and temperature controls and supplementary light.

These differences in the quantity of sap collected (in ml per day) can be seen by examining the following data which are given for different times of the year for each plant:

Time of Study	Ammonium	Nitrates	Time of Study	Ammonium	Nitrates
June-July	10.60	9.40	Sept.-Oct.	3.03	3.06
July-August	9.10	8.70	Oct.-Dev.	0.33	0.50

The results for the dry weight, ash content, and pH are shown in Table 1.

It is obvious from the data in Table 1 that from 40-75% of the dry weight of the sap of pumpkin grown on soil fertilized with ammonium, as compared with the nitrate fertilizer, consists of mineral constituents.

TABLE 1

Dry Elements, Ash, and pH of sap

Expt. No.	Dry elements		Ash		Organic elements as % of dry weight	pH
	mg per 5 ml	% of weight	mg per 5 ml	as % of dry weight		
			Ammonium			
1	20.60	0.41	8.10	39.30	60.70	—
2	25.90	0.52	10.60	41.00	59.00	4.85
3	29.50	0.63	21.75	75.00	25.00	5.46
			Nitrates			
1	21.60	0.43	9.50	43.90	56.10	—
2	13.05	0.26	7.10	54.60	45.40	5.15
3	18.35	0.37	8.17	50.00	50.00	5.50

Inasmuch as the sap contains a significant quantity of nitrates that can be exposed to reduction, we tried several methods to find the most expedient and adequate method of analysis, prior to an actual detailed study of the nitrogen held in the sap.

The question of the effect of the precipitation of the protein on the results of the detection of nitrogen interested us particularly. Tannin and alcohol were used in the process of precipitating the protein. The experiments showed that the character of the precipitate is not evident in the results of the definitely ammoniacal nitrogen of the sap.

Further investigation showed that the precipitate of the protein with tannin and alcohol sharply increased the quantity of known nonprotein nitrogen, as shown in the following data (in mg per 5 ml of sap):

Nitrogen in different forms of precipitate	Ammonium sap, June	Sap of pumpkin, fertilized with $N_{15}H_4NO_3$, May
Ordinary Kjeldahl	0.24	0.53
Ordinary Kjeldahl with the addition of tannin	—	1.65
Nonprotein after precipitating with tannin	—	1.64
Nonprotein after precipitating with alcohol	0.66	0.72
Protein after precipitating with tannin	—	0.09
Protein after precipitating with alcohol	0.07	0.08

TABLE 2

Nitrogen Forms in the Sap of Pumpkin

Expt. No.	Time of sap sample	Fertilizer	mg per 5 ml of sap					% of dry weight					% of ordinary (Kjeldahl + nitrate)		
			by Kjeldahl	NH_3	protein	organic nonprotein	NO_3	ordinary	by Kjeldahl	NH_3	protein	organic nonprotein	NO_3	ordinary	ordinary by Kjeldahl	organic nonprotein	nitrate
1	June – July*	$(NH_4)_2SO_4$	0.24	0.08	0.07	0.09	1.43	1.67	1.18	0.38	0.35	0.43	6.94	8.10	14.37	5.38	85.63
2	June – July*	$Ca(NO_3)_2$	0.28	0.03	0.12	0.13	1.27	1.55	1.29	0.15	0.57	0.60	5.87	7.16	18.06	8.38	81.94
3	Aug. – Sept.	$(NH_4)_2SO_4$	0.33	0.10	0.07	0.16	1.75	2.08	1.27	0.40	0.28	0.62	6.73	8.00	15.86	7.69	84.14
4	Aug. – Sept.	$Ca(NO_3)_2$	0.27	0.07	0.12	0.06	1.25	1.52	2.06	0.57	0.96	0.46	9.57	11.63	17.82	3.88	82.18
5	Sept. – Oct.	$(NH_4)_2SO_4$	0.43	0.09	0.09	0.25	1.70	2.13	1.48	0.32	0.31	0.86	5.86	7.34	20.19	11.73	79.81
6	Aug. – Sept.	$N^{15}H_4NO_3$	0.32	0.12	0.05	0.15	1.28	1.60	1.45	0.55	0.21	0.68	5.82	7.27	20.00	9.40	80.00

* Sap lyophylic dry materials.

The data suggest that the contents of protein nitrogen do not depend on the method of precipitating the protein.

Thus, calculating the precipitate of the normal Kjeldahl nitrogen as the sum of protein and nonprotein nitrogen often can give inexact results in the case of sap analysis. This error is probably the result of the partial recovery of nitrates with tannin and alcohol. Generally, tannin results in strong recovery. In connection with this case, a question is raised about the accuracy and conditions of determining the nonprotein nitrogen in various plant materials which store nitrates. This question demands a special experimental proof.

As a result of the preliminary methodological study, we used the following methods for the study of nitrogenous substances in sap: normal and protein nitrogen were determined by the Kjeldahl method (after precipitation with alcohol). The ammoniacal nitrogen in the sap was determined after precipitation of the protein by distillation in a vacuum at 40°; alkalization was carried on with a mixture of NaOH + borax with pH 9 (1 ml of the mixture for 10 ml of sap). On the basis of the data obtained from this, the difference between the ordinary Kjeldahl nitrogen and the sum of the ammoniacal and protein nitrogen was considered to be the organic nonprotein nitrogen (nitrogen amino acid and amides). The precipitate of the nitrogen nitrates was determined by the Grandval and Lezh methods [14]. The ordinary nitrogen was determined by adding the ordinary Kjeldahl nitrogen and the nitrogen of the nitrates.

The results from six samples of sap taken at different times of the year from plants which had been fertilized with ammonium sulfate, ammonium nitrate, and potassium nitrate are shown in Table 2.

The data shown in Table 2 are used in reaching the following conclusions.

It is quite clear that only an insignificant portion of the nitrogenous substances stored in the sap of pumpkin is made up of organic nonprotein nitrogen; that is, nitrogen amino acid and amides. The main part of the nitrogen of sap is made up of nitrates which, on entering into the vegetative part of the plant, are recovered and are used in the synthesis of amino acids and proteins.

Thus, the overwhelming majority of the inorganic nitrogen assimilated by the roots goes into the leaves for future transformation into organic nitrogenous compounds.

TABLE 3

Ash and Moisture Content, Normal Nitrogen, Carbohydrates, and Phosphorous in Protein Preparations from Sap

Preparation number	Moisture %	Percent of dry weight				Nitrogen, percent of dry weight
		Ash	Nitrogen	Carbohydrates	Phosphorous	
1*	23.0	66.7	3.7	1.3	0.59	11.0
2	0	84.0	2.0	4.1	0.55	8.7
3	0	87.6	2.5	–	–	20.0
4	0	84.7	2.9	7.7	–	18.1

*From nondialyzed sap.

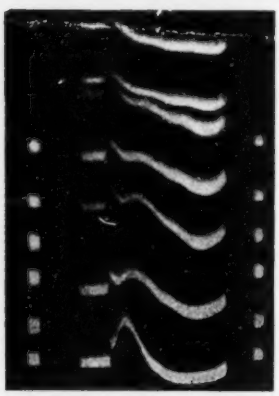


Fig. 2. Sedimentation diagram of a protein preparation from the sap of pumpkin.

It should also be noted that together with the determination of nitrates, we carried out many analyses to determine the nitrites in the sap by means of the sensitive and exact Parker method [15]. We did not discover nitrites in the fresh sap in a single case; these were found only in those cases where the sap was not completely fresh. It appears that the production of nitrites, which is always accompanied by a decrease in the amount of nitrates, is a result of the presence of microorganisms in the sap.

A survey of the data in Table 2 shows that the amount of the nitrogenous substances in the sap practically never changes in relation to the type of fertilizer used in the soil. This is probably due to the fact that in the soil in which the plants were grown, the process of nitrification was so intense that the root system was supplied with an abundance of nitrates. This is probably the reason that the effect of the ammonium salts did not show up in the sap.

The problem in our future work will be the establishment of analagous studies in which the rate of nitrification in the soil will proceed slowly.

The amino acids in the sap of pumpkin were determined by the method of qualitative paper chromotography. The sap was saturated with ethyl alcohol at a ratio of 1:5, the colloid precipitates concentrated in a centrifuge, and the clear alcohol solution evaporated to a small volume in a vacuum at 40° and subjected to chromotography. Part of the experiment was carried on with the lyophilic dry materials of the sap which had been dissolved in a quantity of water equal to the volume of sap; part of the experiment was carried on with fresh sap. The results were identical. The ammonium and nitrate sap in a solution of phenol with phosphate buffer pH 12 [16] was found, using the Leningrad "slow" No 2 paper test, to contain the following amino acids: aspartic and glutamic acid (very weak spots), tyrosine, methionine, and also amides, asparagine (weak spot) and glutamine with alanine (generally strong spot).

When the above was carried out in a solution of M-kresol with phenol and a borate buffer pH 8.3 (in the relationship 4:2:1), spots for glutamic acid, glutamine (with alanine), valine, leucine, and methionine (Fig. 1) were clearly visible. There was a stronger color on nearly all chromatograms than for the glutamine spot. But because on the application of the above solutions, glutamine and alanine are placed on the chromatogram together, the intensity of the color spots can be explained by the presence of those, and also other, compounds. In order to clear up this question, chromatograms of the hydrolized sap were made.

Hydrolysis was carried on in the following manner: an alcohol solution of the sap was dried in a vacuum, the dry residue was dissolved in 3 ml of water (pH of the solution was approximately 6) and hydrolized in a

boiling water bath for an hour, during which the glutamine was completely broken down. Then the hydrolized solution was again dried and dissolved in 0.5 ml of water. For chromatography, phenol and M-kresol were used for dissolving. It appeared that the intensity earlier observed for the glutamine was greatly decreased after development.

It should be noted that the intensity of the color spot of glutamic acid after hydrolysis is not proportional to the quantity of the glutamine which disappears, which showed that glutamine when submitted to hydrolysis is converted not only into glutamic acid, but also into pyrrolidonic acid, which is not identified by this method.

On the chromotograms of the sap after hydrolysis there was a weaker spot for glutamine, which corresponded exactly to that for alanine. This was especially clear when alanine was developed on chromotograms where the solution used was M-kresol-phenol-borate buffer with pH 8.3 (see Fig. 1).

In this way, we identified the following amino acids in the ammonium sap, as well as in the nitrate sap: aspartic and glutamic acids, asparagine, glutamine, valine, leucine, methionine, alanine, and tyrosine. The strongest spot in the ammonium sap, as in the nitrate sap, was the glutamine.

Sap Protein

The addition of alcohol or several drops of a saturated solution of tannin to sap forms a noticeable amount of a flaky precipitate. It can be assumed that this flaky precipitate is protein.

The first portion of sap was discarded and then the surface cut was dried with a piece of filter paper; this was done to remove the possibility of cell protein from the injured cells of the surface cut on the pumpkin stem from falling into the sample. The collection of 500 ml of sap was refrigerated in a mixture of dry ice and alcohol and lyophilized. Then the dry residue was dissolved in 100 ml of double-distilled water and the solution was subjected to dialysis with distilled water under refrigeration until the ninhydrin reaction in the water completely disappeared. Dialysis usually continued for 48 hours.

After dialysis, five times the volume of distilled 96% ethyl alcohol was added to the solution for precipitation of the protein. A small, white flaky precipitate was obtained on addition of alcohol, and this was subjected to a centrifuge and washed with alcohol. The precipitate received was agitated in water (the precipitate was not completely dissolved), refrigerated in a mixture of dry ice and alcohol, and then lyophilized. Preparation 1, in contrast to preparations 2, 3, and 4, was obtained by precipitating protein in fresh nondialyzed sap, followed by lyophilisis of the precipitate. About 100 ml of a light, white preparation of protein was obtained from 500 ml of sap.

The preparation thus obtained gave a series of characteristic reactions on the protein: Millon, xanthroprotein, and biuret. A Molisch reaction on carbohydrates was weak.

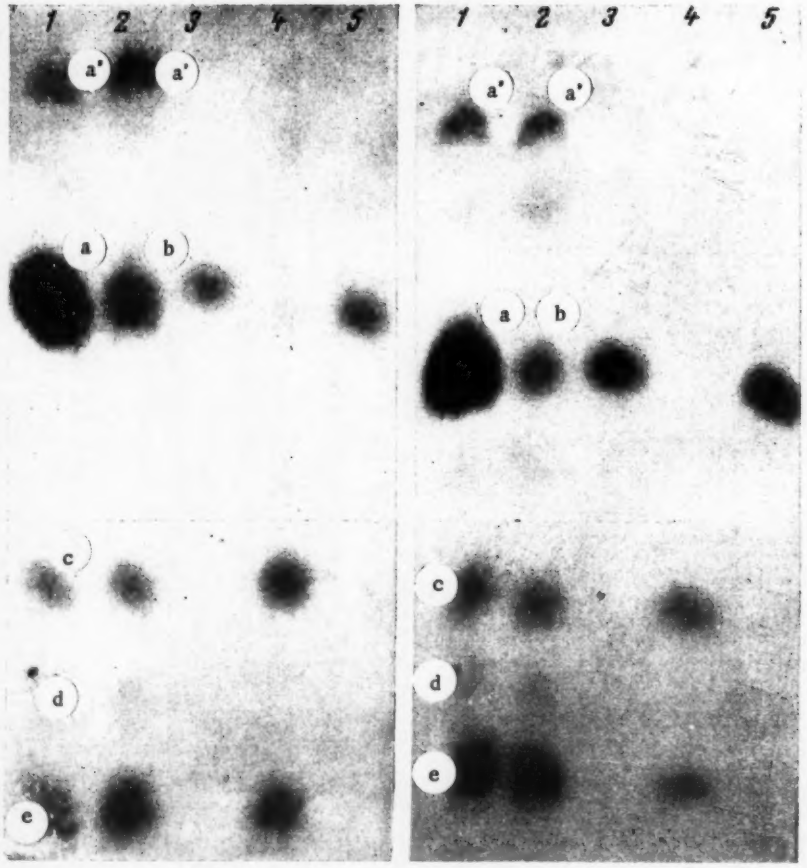
In the protein preparations, water, ash, ordinary nitrogen and phosphorous were identified by the Fisk and Subbaro methods. Noting that the Molisch reaction on carbohydrates was positive, the quantity of the carbohydrates in the protein preparations was determined with anthranone according to Trevelyan and Harrison [17].

The data obtained are shown in Table 3.

From the data given in Table 3, it can be seen that on dialysis of sap, substances were obtained from the sap which indicated extreme hygroscopicity in the protein preparations. The protein preparations contained a small quantity of carbohydrates and phosphorous and a larger quantity of ash. Keeping in mind that the sap from which preparations 2, 3, and 4 were obtained was subjected to a preliminary dialysis, it must be assumed that the ash consists of substances not drawn off by dialysis.

Analysis of a quantity of ash by the half-strength spectrum method [18] showed that the ash contained a significant quantity of silicon and calcium. The number of other mineral elements was quite small.

The amount of ash (%) in protein preparation No. 2 from the sap of pumpkin showed the following: silicon, more than 10%; calcium, more than 10%; magnesium, approximately 5%; iron, approximately 1%; aluminum, approximately 1%; copper, 0.5%; zinc, 0.5%; barium, 0.3%; antimony, 0.1-0.5%; chrome, 0.03%; manganese, 0.01%; and titanium, 0.001%.



A B

Fig. 1. Chromatogram of "ammonium" sap (A) and "nitrate" saps (B). Solution M-kresol-phenol-borate buffer (4 : 2 : 1); pH 8.3.
1)Sap, before hydrolysis: a' – glutamic acid, a – glutamine (with alanine); b – valine, c – methionine, d – leucine; 2) sap after hydrolysis: a' -glutamic acid, b – alanine, c – valine, d – methionine, e – leucine. Amino acid "spots" 3) alanine; 4) valine, leucine; 5) glutamine.

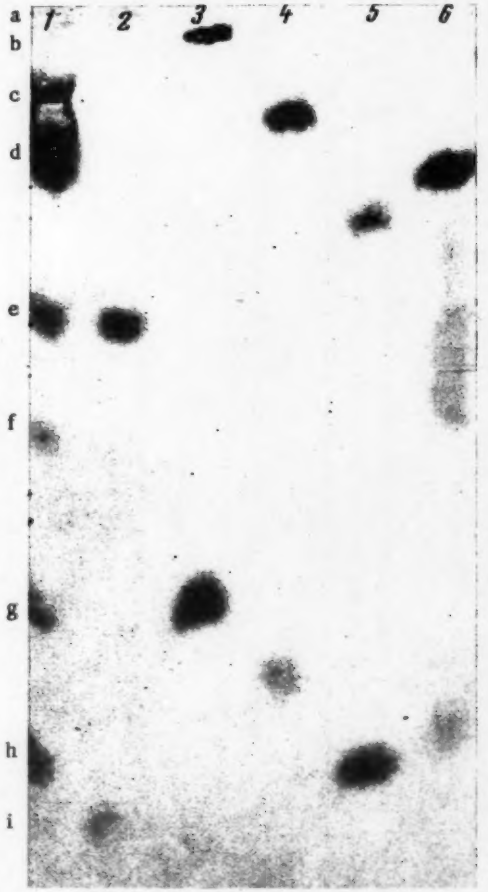


Fig. 3. One-dimensional chromatogram of the hydrolysis of sap protein. Solution: M-kresol-phenol-borate buffer, pH 8.3.
1) Protein hydrolysis; amino acids of hydrolysis; a – aspartic acid, b – glutamic acid, c – serine, d – glycine, e – alanine, f – tyrosine, g – valine, h – leucine, i – phenylalanine. Amino acid "spots"; 2) aspartic acid, alanine, phenylalanine; 3) glutamic acid, valine, arganine, proline; 4) serine, methionine; 5) leucine, threonine; 6) tyrosine, tryptophan, glycine.

We also determined the amount of calcium with trilon [19]. It appeared that the calcium made up 35% of the ash elements of the preparation. Therefore, the silicon made up about 55% of the ash by weight.

The data did not permit us to resolve the question of the connection of silicon and calcium with the sap protein. It is possible that silicon and calcium located in the sap are not connected with the protein, but are connected with those salts which did not show up in the dialysis in the strength of their colloid properties. The determination of the molecular weight of preparation No. 1 shows that the sap protein appears fastest with albumen.*

The average molecular weight of the preparation was determined by measuring the sedimentation equilibrium. The determination was carried out in a Svedberg ultracentrifuge at 60,000 revolutions per minute. For measurement a portion of the preparation was precipitated in a phosphate buffer with pH 9.18 + 10% NaCl; the concentration of protein in the solution was exactly 1%.

The experiment continued for 68 minutes. Observation of the progress of the sedimentation was carried on by means of the Swenson oblique aperture; photographs were taken every 10 minutes.

In Fig. 2 a sedimentation diagram of the sap protein is shown.

On the reduced photograph, three independent sedimentation compounds are seen. The average molecular weight, as calculated by the Svedberg formula using the data obtained, was 100,000.

Then a preliminary investigation of the amino acid concentration of the preparations was carried out on one and two dimensional paper chromotography. Hydrolysis of protein (preparation No. 2) was carried out in the absense of air in a sealed glass tube with 7.2N HCl for six hours in a dessicator at a temperature of 150°. Then an ampoule was refrigerated, opened, contents put in a small procelain dish and the HCl was completely eliminated in the vacuum drier over KOH and P_2O_5. The residue was dissolved in a small quantity of water and used for chromotography. Two-dimensional chromatograms were made by the Levy and Chung method [20]. Sixteen amino acids were found. The identification of a portion of the amino acids was done by one-dimentional paper chromotography with a solution of M-kresol-phenol-borate buffer of pH 8.3.

The following amino acids were found on the one-dimensional chromotogram: (Figure 3) aspartic acid, glutamic acid, serine, glycine, alamine, tyrosine, valine, leucine, phenylalanine.

The data obtained made it possible to assume that a small quantity of protein of the albumen type is located in the sap, and it is possible that it is connected with compounds of silica and calcium. However, the question of the role of this protein needs further experimental work.

SUMMARY

Investigation of the forms of nitrogenous compounds in the sap of young pumpkin plants grown on soil fertilized with nitrates and ammonium salts, was carried out.

From 40 to 75% of the dry substance of sap consists of substances found in burned ash. The greater part, from 80 to 84%, of the nitrogenous substances of sap are nitrates. Nitrogen, as determined by the Kjeldahl method consists of from 14 to 20% of ordinary nitrogen and the organic (nonprotein nitrogen amino acids and amides) together only 5 to 12% of the ordinary nitrogen of sap. Nitrites are not found in sap.

With the help of one-dimensional paper chromotography, using a solution of phenol with a phosphate buffer of pH 12 and M-kresol with phenol and borate buffer of pH 8.3, the presence of the following free amino acids and amides in the sap was established: aspartic and glutamic acid, tyrosine, methionine, alanine, valine, leucine, asparagine, and glutamine. Especially intense in color were the ninhydrin spots of glutamine.

On adding a solution of tannin to the dialyzed sap, or on saturating it with alcohol, a noticeable colloid precipitate was formed, giving a protein reaction and having an extremely high ash content. In the hydrolysate of the preparation obtained in this manner, sixteen amino acids were discovered with the help of two-dimen-

*The molecular weight of the protein preparation was determined in the Institute of High Molecular Compounds of the Academy of Sciences USSR, in Leningrad by S. Ya. Frenkel' and T. I. Smirnov, to whom we extend our thanks.

sional paper chromotography. The following amino acids were found in the hydrolysate with the aid of one-dimensional chromotography: aspargic and glutamic acids, serine, glycine, alanine, tyrosine, valine, leucine, and phenylalanine.

The data obtained led to the conclusion that a small quantity of protein is stored in the sap and it is possible that it forms a stable complex with the salts of silica and calcium. The study of this protein is the goal of our next investigation.

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THE INTERRELATIONSHIP OF FOLIAR AND ROOT MINERAL NUTRITION IN PLANTS

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Growth experiments performed by us in 1953-1955 led to the conclusion that the closest interrelationship exists between foliar and root mineral nutrition in plants.

In the experiments the results of which are reported below 10 plants of spring wheat, variety Artemovok, were grown on exhausted chernozem soil in pots containing 10 kg of absolutely dry soil. The soil contained per 100 g 2.3 mg N in the easily hydrolyzable form and 7.8 mg of mobile phosphorus as P_2O_5. The comparatively poor quality of the soil made it possible to created different nutrient environments the addition of fertilizers. The plants were grown in nutrient environments which we arbitrarily designated $N_1P_1K_1$, $N_1P_3K_3$, $N_3P_1K_3$, where the figure 1 or 3 after the nutrient element indicated one or three doses of the corresponding fertilizer. One dose was composed of: NH_4NO_3 – 2 g, superphosphate – 2 g, KCl – 1 g. Soil moisture content was routinely maintained within 60-70% of the saturation level. For foliar feeding a nutrient solution was used which contained, per liter: $CO(NH_2)_2$ – 4.5 g, superphosphate – 12.5 g, KCl – 3.5 g. Other forms of nitrogen – $(NH_4)_2CO_3$, NH_4NO_3, $Ca(NO_3)_2$ – applied in an initial concentration corresponding to a 0.45% solution of urea, were tested in one of the experiments. The soil surface was protected from the nutrient solution during foliar feeding.

Attention was directed principally to the following questions: to what extent foliar nutrition supplements root nutrition, how it alters the dynamics of accumulation of mineral nutrients by the aerial organs, how the final yield depends on such alterations. Seven-11 days after foliar application which was carried out during shooting or heading, the main stem and lateral branches of control and experimental plants were compared with respect to relative and absolute contents of total nitrogen, protein nitrogen, phosphorus and potassium; these were also determined in stems prior to foliar application of the nutrient solution. Fresh plant samples taken for analysis were carefully washed with distilled water. Analyses were performed on replicate plants and the remaining plants were used in yield evaluation. Total nitrogen was determined according to the Kjeldahl method, protein nitrogen by Barnshtein's method, phosphorus by a colorometric method, and potassium by flame photometry.

In Table 1 we see that in plants grown on soil with the nutrient composition $N_1P_1K_1$ to which foliar application is made in the shooting stage the main stem contains, in all cases, at the time of heading (11 days after application) an evan smaller percentage of total nitrogen, phosphorus and potassium than in control plants. With respect to lateral branches, the effect is the reverse – there is an increase in the percentage of nitrogen and phosphorus, as a result of foliar application; this is true for potassium only in the case of application of NPK. The decrease in the quantity of nutrients per unit dry weight in the main stem may be due to an increased rate of its growth, to a "dilution" of the mineral elements by carbohydrates. The analytical data, however, indicate that the absolute content of N, P and K is also higher in lateral branches and lower in main stems 11 days after foliar application in comparison with control plants of the same age.

In Table 2 we see that foliar application of N, P and NPK resulted in a 1.6-2.7-fold increase in mineral content of lateral stems and a 15-50% decrease in main stems during the period of growth, "third internode-heading" (with the single exception of an increase in the main stem with application of phosphorus). The dry weight increment after application also varied with individual stems; where urea was applied it was 7.18 in the main stem as compared with 7.49 g for the control and 3.57 g in lateral stems as compared with 2.17 g for the control (data from plants in one pot).

TABLE 1

Effect of Foliar Application of Nutrients in the Shooting Period (Third Internode) on the Percentage Composition of N, P, and K in the Stems of Spring Wheat at the Heading Stage ($N_1P_1K_1$)

Plants analyzed	Main stem				Lateral stems			
	N		P_2O_5	K_2O	N		P_2O_5	K_2O
	total	protein			total	protein		
Controls	2.23	1.95	0.59	2.37	2.52	2.10	0.63	2.71
Foliar application N*	2.09	1.83	0.52	2.19	2.60	2.24	0.68	2.57
» P	2.18	2.07	0.51	2.08	2.62	2.37	0.65	2.61
» NPK	2.18	1.95	0.54	2.22	2.65	2.35	0.68	2.88
Controls, on the day of application	3.85	2.47	0.54	3.04	3.62	2.75	0.56	5.13

TABLE 2

Effect of Foliar Application of Nutrients in the Shooting Period (Third Internode) on the Accumulation of N, P, and K in the Stems of Spring Wheat (soil nutrient composition $N_1P_1K_1$; increase in N, P, and K in mg for 10 plants during the period of growth, "third internode-heading")

Plants analyzed	Main stem				Lateral stems			
	N		P_2O_5	K_2O	N		P_2O_5	K_2O
	total	protein			total	protein		
Controls	52	110	48	130	39	36	15	24
Foliar application N*	25	86	36	96	79	72	26	55
» P	57	138	39	98	79	78	24	56
» NPK	44	109	41	107	76	74	25	65
Foliar application $(NH_4)_2CO_3$	53	116	—	—	71	66	22	90
» NH_4NO_3	34	102	—	—	41	39	18	57
» $Ca(NO_3)_2$	7	78	—	—	56	50	20	63

A reasonable explanation of these facts is that foliar nutrition exerted an influence on the nutrition of aerial organs by roots having caused a redistribution of nutrients flowing from the root system: their movement into the lateral branches was stimulated, and into the main stem suppressed. This assumption also serves to explain, for example, the fact that after application of urea there was not only more nitrogen but also more phosphorus and potassium in the lateral branches; in the main stem, however, nitrogen content was decreased even after application of urea, and phosphorus content even after application of superphosphate.

The data in Table 3 indicate that even with a second foliar application to spring wheat on soil with the nutrient composition $N_1P_1K_1$ there is usually an increase in the mineral nutrient content of lateral stems and a decrease in the main stem, although these changes were not as marked as after the first application; exceptions could be found.

Of possible interest is the extent to which folier application to plants on $N_1P_1K_1$ soil altered the absolute content of N, P, and K in the aerial portion of the plant considered as a whole. Suitable calculations may be made on the basis of data in Table 2 and 3. Most typical data are those involving application of urea and superphosphate. In the shooting stage, the nitrogen content was increased after application, particularly of phosphorus. The phosphorus level, however, remained almost at the level of the control, even in the case of application of superphosphate. If the influence of foliar nutrition on root nutrition were not taken into account,

TABLE 3

Effect of Foliar Application of Nutrients in the Heading Stage on the Accumulation of N, P, and K in Stems of Spring Wheat. (soil nutrient level $N_1P_1K_1$; increase in N, P, and K in mg for 10 plants during the period of growth, "heading-end of flowering")

Plants analyzed	Main stem				Lateral stem			
	N		P_2O_5	K_2O	N		P_2O_5	K_2O
	total	protein			total	protein		
Controls	45	53	28	-1*	51	53	21	52
Foliar application: N	41	44	28	-4	81	77	26	71
" " P	22	17	30	-11	75	69	27	67
" " NPK	56	33	32	3	63	59	23	45

* A number preceded by a minus sign (-) indicates a decrease in K_2O content.

this would seem improbable: phosphorus application had no effect on phosphorus content of the aerial organs, while it markedly increased nitrogen accumulation. Application of superphosphate at the heading stage did not improve nitrogen nutrition, but the phosphorus content was increased by phosphorus as well as nitrogen application.

Foliar nutrition in the shooting period involved four forms of nitrogen (with the same initial concentration of nitrogen). These compounds are arranged in descending order according to their effect on nitrogen accumulation in the stems under the conditions of the experiment: $(NH_4)_2CO_3$, $CO(NH_2)_2$, NH_4NO_3, $Ca(NO_3)_2$; while the reduced form of nitrogen exerted a promoting effect, the oxidized form had an inhibitory effect. Application of saltpeter brought about a decrease in nitrogen content at the heading stage compared with the controls, not only in the main stem but also in the aerial organs as a whole; even in this case, however, accumulation of N, P, and K in the lateral branches was stimulated markedly. Ammonium carbonate had an extremely favorable effect, although a considerable part of the nitrogen was not incorporated into leaf tissue because of the degradation of this compound to NH_3, CO_2 and H_2O with the evaporation of ammonia from the leaf surface.

Nitrogen (urea) application to plants on soil with a nutrient composition $N_1P_3K_3$ exerted a lasting positive effect on the accumulation not only of nitrogen but of phosphorus and potassium in the main and lateral stems. At the end of the flowering period the plants supplied with nutrients through the leaves contained in the main and lateral stems as compared with the controls, respectively: total N—1.68% against 1.39% and 1.76% against 1.50%; protein N—1.60% against 1.25% and 1.59% against 1.30%; P_2O_5—0.62% against 0.54% and 0.61% against 0.56%; K_2O—2.55% against 2.08% and 2.81% against 2.60%. For a similar comparison of absolute dry weight of 10 plants we have: 18.75 g against 18.35 g and 34.50 g against 33.97 g; in conclusion, not only the percentage content but also the absolute content of N, P, and K in the stems is increased by nitrogen application (Table 4).

Foliar application of superphosphate to plants on soil with a nutrient composition $N_3P_1K_3$ was as a whole considerably less effective with respect to mineral nutrition; a two-fold application in the shooting period resulted in a slight increase in phosphorus level in the aerial organs, but had not marked effect on the accumulation of nitrogen and even depressed potassium flow from the roots; after a third application the entrance of nitrogen into the aerial organs was markedly stimulated, but again there was a depressed potassium nutrition.

In all cases it may be stated that an increase in grain yield associated with foliar application of nutrients is related to a stimulation of nutrition of the aerial organs by the roots, and that a decrease is related to its suppression. Under conditions of limited water supply, foliar application suppressed root nutrition in spring wheat and at the same time resulted in a decrease in yield. In growth experiments carried out in 1954-1955, grain yields of spring wheat, supplied with nutrients through the leaves and grown under conditions of low and high soil moisture content, expressed as percent of the control value, were, respectively, for several cases: 88.3% and 104.9%; 86.9% and 127.9%; 94.9% and 108.6%; 81.1% and 114.4%; for crude protein yield of the grain:

TABLE 4

Effect of Foliar Application of Nitrogen to Plants on $N_1P_3K_3$ Soil and of Phosphorus Application to Plants on $N_3P_1K_3$ Soil on the Accumulation of N, P, and K in the Aerial Organs of Spring Wheat (increase of N, P, and K in mg for 10 plants during the indicated growth periods)

Analyzed plants	"2nd internode – heading" (after two applications)				"Heading – end of flowering" (after third application)			
	N		P_2O_5	K_2O	N		P_2O_5	K_2O
	total	protein			total	protein		
Controls, $N_1P_3K_3$ soil	132	173	91	379	38	26	32	46
Foliar application, $N_1P_3K_3$ soil	204	196	112	457	68	121	37	101
Controls, $N_3P_1K_3$ soil	389	300	93	628	81	95	43	50
Foliar spplication, $N_3P_2K_3$ soil	386	340	99	606	126	111	50	27

89.6% and 106.4%; 87.8% and 137.2%; 96.5% and 112.2%; 86.6% and 119.6%. It should also be mentioned that in our field experiments foliar applications to wheat and sugar beet had the most unfavorable effects in dry years.

On $N_1P_1K_1$ soil with a moisture content of 70%, a depression of nutrition of the main stem from the roots resulting from foliar application of nutrients was correlated with a decrease in yield, and an increased nutrition of lateral branches from the roots was associated with an increased yield. Foliar application during the shooting period was followed by an especially marked alteration in root nutrition as well as in grain yield of the different stem types.

On $N_1P_3K_3$ soil foliar application of nitrogen resulted in a marked improvement in root nutrition of the main stem as well as the lateral branches, and also in an increased grain yield. On $N_3P_1K_3$ soil application of phosphorus at the same times altered the level of mineral (nitrogen, phosphorus) nutrition of the aerial organs to a lesser extent and over a shorter period of time, and even resulted in a depression of potassium nutrition; in spite of a certain increase in grain content of the head, yield was decreased because of poor ripening of the grain.

Of course, we have not found complete agreement between changes in yield and mineral nutrition in the course of these detailed comparisons. In order to fully understand this relation, it is necessary not only to make single comparisons of control and experimental plants with respect to the content of three nutrient elements in the stems a certain number of days after foliar application of nutrients, but also to study the effect of foliar application on the whole subsequent history of root nutrition and on the over-all dynamics of metabolic processes the end result of which is grain production.

At the present time, the amount of material which has been accumulated on the effect of foliar application of mineral nutrients on yield of agricultural crops is considerable but extremely contradictory. Some authors report uniformly large increases in yield, and others claim that this method is totally ineffective. A number of investigators assert that foliar application of mineral nutrients in small doses is not less but even more effective. Morgatskii [1] notes that 30-50 kg/hectare of mineral fertilizer has the same effect on sugar beet yield as an amount one-third as great. In the experiments of Mednis [2], quite small doses, 1-2 kg/hectare, increased grain yield of cereal grasses 4.6 cwt./hectare. Webber [3] notes that foliar application of urea to fruit trees increased yield and improved fruit quality when made in moderate quantity (0.5% solution) and when the soil contained adequate reserves of nitrogen.

Large doses of mineral nutrients result in plant injury and disruption of metabolic processes. It may prove that the quantity of nutrients which we introduce into the aerial organs through the leaves is not as important as the quantity which enters the aerial portions as a result of subsequent increase in the level of root nutrition. In that case we should, obviously, apply nutrients to the leaves in small doses, knowing that our chief purpose

TABLE 5

Effect of Foliar Application of Nutrients to Spring Wheat on Grain Yield and Crude Protein Content of Grain (data for plants in one pot, in g)

Nutrient composition of soil	Soil moisture content, in % of saturation value	Conditions of foliar application: time of application, as stage of development	Conditions of foliar application: nutrient element applied	Main stem: grain	Main stem: crude protein	Lateral branches: grain	Lateral branches: crude protein
$N_1P_1K_1$	70	Control	—	13.17	2.27	3.15	0.60
		Shooting stage	N	11.81	2.09	6.29	1.14
			P	11.35	1.79	6.04	0.99
			NPK	12.72	2.21	6.86	1.22
		Heading stage	N	13.13	2.25	4.33	0.84
			P	12.38	2.11	4.53	0.76
			NPK	13.46	2.37	3.66	0.68
$N_1P_3K_3$	70	Control	—	10.30	1.57	10.05	1.58
		Shooting stage + heading stage	(N+N)+N	12.79	2.24	14.05	2.32
$N_3P_1K_3$	70	Control	—	11.89	2.52	9.41	1.74
		Shooting stage + heading stage	(P+P)+P	11.23	2.27	9.07	1.61
$N_1P_1K_1$	30	Control	—	8.57	1.77	—	—
		Heading stage	NPK	7.57	1.58	—	—

is to enhance nutrient flow from the root system. This, of course, is not to deny that there may be an increase in the primary role of nutrients applied through the leaves to plants growing on soils poorer in mineral nutrients. Obviously, foliar application may also promote a more effective utilization of nutrients entering the roots both before and after application. Moreover, we may directly satisfy the plants microelement requirements by foliar application, but it should be assumed that, in this case as well, the level of root nutrition is altered with respect to macroelements; from this point of view investigations concerned with foliar application of growth stimulators are also of interest.

It should be pointed out that the question of the interrelationship of root and foliar mineral nutrition is not a new, unexpected development. It stems from the very nature of the work of the Soviet school of physiologists who study the living organism as a single entity. Such studies as those of D. A. Sabinin, A. L. Kursanov, N. G. Potapov, I. I. Kolosov, O. F. Tueva, E. I. Ratner and others were concerned with root nutrition and problems of the metabolic interrelation of the aerial organs and the root system. In truth the problem of foliar nutrition was developed at first somewhat independently, and also as a definite offshoot of these investigations. For example, N. G. Potapov had already shown in the prewar years (see Sabinin [4]) that the absorptive activity of the root system is dependent on the outflow of assimilates (products of photosynthesis). In the prewar years Komissarov [5], Ustenko [6] and Rikhter and Vasileva [7] established, moreover, that the rate of photosynthesis could be increased considerably by foliar application of nutrients. Thus, the investigations of N. G. Potapov and the other workers mentioned on photosynthesis had already suggested that the existence of a relation between root and foliar nutrition was entirely possible. In prewar experiments of Levin [8] foliar application of potassium exerted a specific effect on oat plants, causing a markedly enhanced root growth, while nitrogen and phosphorus application had its main effect on the growth of aerial organs. But these facts were also largely unnoticed. Many similar examples may be adduced.

According to present views, mineral nutrition, an active physiological process, is determined by the vital activity of the root system as well as that of the aerial organs. Obviously, the increase in rate of synthetic processes in the plant as a result of foliar application of mineral nutrients, which is first manifested in the aerial organs — increase in photosynthesis, protein synthesis, growth, etc. is of crucial significance in the enhancement of root nutrition.

As was shown, foliar application of nutrients to plants on $N_1P_1K_1$ soil produced distinct, even opposing effects on the main stem and the lateral branches. Krasovskaya and Kumakov [9] and Kolosov and Shaldenkova [10] showed that primary and secondary roots of cereal grasses may provide all the stems of the plant with water and mineral nutrients equally; their redistribution occurs, according to Kolosov and Ukhina [11], upon passage

through the tiller nodes. These investigators report that the quantity of nutrients taken into various stems from separate groups of roots is determined, among other things, by the rate of growth and development of these stems, by their requirements in other words.

On the basis of their experiments Kursanov and Zaprometov [12] and Afanas'eva [13] conclude that adsorption processes play a large role not only in initial uptake but also in subsequent transport of materials. The direction of this transport is related to the increasing adsorptive capacity accompanying metabolic activity in living tissues (a result of conversion of materials into an immobile form, and also of the synthesis of new adsorbing components or the qualitative alteration of already existing components).

In our experiments on $N_1P_1K_1$ soil, nutrients entering the lateral branches through foliar application markedly enhance the synthesis of proteins, as shown by the accumulation of protein nitrogen following application (Tables 2 and 3). In the enhancement of root nutrition of plants as a result of foliar application of nutrients, the synthesis of proteinaceous adsorbers, which largely determine the active role of individual stems and the plant as a whole in the uptake of nutrients, is of great significance. A decrease in the uptake of minerals from the roots by the main stem does not necessarily imply an adverse primary effect of foliar application of mineral nutrients on its synthetic processes although, even when there is a promoting effect, it is obviously smaller than in lateral stems. A considerable increase in the uptake of minerals from the roots by secondary stems as a result of foliar application of nutrients under conditions of comparative deficiency of soil nutrients could lead indirectly to a decline in the level of uptake of nutrients from the roots by the main stem and also to a subsequent suppression of its synthetic processes. In the course of 11 days following foliar application of nutrients during the shooting period, the total amount of N, P, and K in the main stem had markedly decreased but, in spite of this, the amount of protein nitrogen had not yet diminished after application of NPK, and after application of P it had even exceeded the control; after N application the main stem contained less protein nitrogen (Table 2). These data confirm the fact that with phosphorus in the nutrient solution protein synthesis in the main stem was increased as a direct result of foliar application. Unfortunately, determinations immediately after application were not made, and therefore the primary effect of urea cannot be assessed. For the solution of a number of problems isolated foliar applications to individual stems of a cereal grass should be made.

Foliar applications under conditions of limited water supply and high temperature suppressed root (NPK) nutrition of spring wheat; a similar alteration in protein synthesis was correlated with this in our experiments. In one of the experiments at 30% soil moisture content (yield data in Table 5), plants sprayed with NPK in the heading stage accumulated in the aerial organs during the period "heading– end of flowering", as compared with the controls: protein N 4.3 mg against 28.0 mg, total N 15.0 mg against 31.0 mg, P_2O_5 3.7 mg against 9.5 mg, K_2O 7.1 mg against 32.0 mg (based on one growing pot). In this case, obviously, flow of nutrients from aerial organs may be involved. On $N_1P_3K_3$ soil foliar application of urea markedly enhanced uptake of nutrients from the roots and protein synthesis in both the main stem and the lateral branches (Table 4).

In the detailed comparison of quantitative changes in protein synthesis and mineral nutrition of the aerial organs of plants after foliar application, the greatest correlation was observed for nitrogen nutrition (indeed, nitrogen in the last analysis is utilized in protein construction), while for phosphorus and especially for potassium nutrition, correlations were small or even negative in some cases. But this is only indicative of large qualitative differences in metabolism, including protein metabolism, which it is impossible to express in simple quantitative terms. Obviously, it is not even so much the quantity of protein which is altered as its capacity for selective adsorption of certain substances. Of course, the significance of other adsorbers of mineral nutrients as well as the overall significance of many other aspects of metabolism should also be taken into account.

Photosynthesis is the first link in a chain of biological synthesis involving conversions of matter and energy. Nichiporovich [14] came to the conclusion, on the basis of experimental results, that not only carbohydrates but also other compounds, such as proteins and amino acids, are formed in the chloroplasts by photosynthesis; the nature of the photosynthetic products depends in large degree on the age of the leaves. Mature leaves and plants synthesize only carbohydrates, while young leaves and plants synthesize in addition large amounts of protein or its precursors. Foliar application of mineral nutrients should obviously be regarded as a procedure which may alter not only the photosynthetic rate but also the nature of the photosynthetic product. Sabinin [15] related photosynthetic products such as carbohydrates to "passive growth", and proteins and substances which are in general characterized by the ability to promote the de novo formation of molecules like themselves to "active growth". He states that the increase of dry matter over a long period would be considerable if the increase in photosynthetic rate were related not so much to carbohydrate formation as to protein formation.

The interrelationship of foliar and root nutrition as mediated by photosynthesis obviously involves both its main components — protein and carbohydrate synthesis. The active adsorption and subsequent conversion of mineral nutrients by the root system depends directly on the flow to it of organic substrates, primarily carbohydrates. On the other hand, the active role of the aerial organs, individual stems, depends to a great extent on the formation by synthetic processes, including photosynthesis, of proteinaceous adsorbers. Obviously, flow of photosynthates to the roots must be largely from the older leaves and stems, where carbohydrate synthesis predominates, and flow of mineral nutrients from the roots must be largely to young leaves and stems, in which protein synthesis predominates (clearly the distinct effects of foliar nutrition on the main stem and the lateral stems which were observed experimentally should be considered from this point of view).

In other experiments carried out just after those described in this paper, foliar application to spring wheat had a marked effect on the flow of soluble sugars to the roots while at the same time it induced change in photosynthetic rate (the total sugar content in the roots a few days after application had increased 70-80% in a number of cases). In connection with this flow of organic materials to the root system, potassium was found to play a specific role. The experiments also showed that foliar application markedly alters the mineral nutrient level of the root system as well as the cycling of mineral elements between the subterranean and aerial parts of the plant. These experimental data require separate consideration.

SUMMARY

The purpose of the present vegetative experiments on winter wheat was to investigate to what extent foliar application supplements root nutrition, to examine how it changes the mechanism of accumulation of the chief elements of mineral nutrition (NPK) in the overground plant organs and how these changes influences the final crop yields.

The results obtained show that nutrition by foliar application is not only an additional channel of nutrition, but also a means of regulating root uptake. The changes in the level of mineral nutrition of the overground organs of the plant are due not so much to the foliar adsorption itself as to the effect of the latter on the uptake of nutrients by the root system. The positive effects of foliar applications on root nutrition can become negative, depending on the moisture level, on the amount of nutrients in the soil and on the form of mineral nutrients applied, especially nitrogen.

An increase in the yield of winter wheat in the case of foliar application was related to enhancement of root nutrition; a reduction of the yield coincided with a drop in uptake by roots. Foliar application should be considered as an agrotechnical method of raising the effciency of fertilizers introduced into the soil in a particular environment and as a method of raising the efficiency of utilization of soil fertility. Foliar application of course should not be employed if it decreases the uptake of nutrients from the soil.

The effect of foliar application of mineral nutrients on the flow of minerals from the roots and on yield varies in degree in individual stems of cereal grasses, and may even be adverse.

Enhanced root nutrition following foliar spraying increases synthetic processes in the plant, e.g., photosynthesis, growth, synthesis of protein substances.

A more profound study of the relationships between mineral nutrition of the plants by leaves and the root system should permit physiologists to gain a better understanding of the nature of nutrition by foliar application and to indicate methods of its most effective usage. Such investigations are necessary for a study of other physiological problems, e.g. the means by which the plant organism acts as a unit and how coordination of metabolism in the aerial parts and root system is attained.

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THE TRANSPORT FORM OF CARBOHYDRATES IN PUMPKIN PLANTS

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Chemical analysis of isolated conducting elements of beet [1] and cotton [2] indicate a predominance in them of sucrose. This disaccharide also makes up an overwhelming proportion of the dry matter in the phloem exudate of a number of woody plants [3]. Recently the predominance of sucrose in phloem exudate was demonstrated by partition chromatography [4, 5]. These investigations provided the basis for considering sucrose the transport form of carbohydrates in plants. A study of radioactive substances flowing from the leaves into the stems of various plants in comparatively short time intervals established the predominance of radioactive sucrose in stems of soybean [6], conducting elements of sugar beet [7], tomato stems [8], and the cortex of grape vines [9]. Investigation of the composition of materials translocated in graft scions of Jerusalem artichoke on sunflower showed that sucrose is the sole transport form in both components although they are basically different with respect to carbohydrates in other tissues [10].

Studying the translocation of organic substances in pumpkin plants with radioactive carbon, we found it appropriate to follow the conversions of the sugars being translocated as well.

To this end, the distribution of radioactive carbon among individual substances or groups of substances in the stem 30 and 60 minutes after the beginning of outflow of assimilates from a leaf exposed to $C^{14}O_2$ for 20 minutes was studied.

Separation of radioactive substances in an alcohol extract was accomplished by paper partition chromatography in a developing solution of butanol – acetic acid – water in the ratio of 4: 1: 5 (B – A – W).

Observation of changes in the radioactivity of components of an alcoholic extract of the stem with varying periods of outflow made it possible to distinguish the primary form of carbohydrate appearing in the conducting system from the products of its further conversion. These experiments were performed using young 20-25-day-old plants as well as 50-day-old plants with greatly elongated shoots.

Results of one experiment are presented in Table 1.

These data show that with a short period of outflow, the mono- and disaccharides account for 20-28% of the total radioactivity of the alcoholic extract, with the major part being concentrated in sucrose.

With a prolonged period of outflow the radioactivity of the mono- and disaccharide fraction increases to 38-44% of the total radioactivity. One of the causes of the later entrance of radioactive sucrose and monoses into stems may be that they move less rapidly through the conducting elements of pumpkin than substances which originally accounted for 70-80% of the radioactivity of the alcoholic extract.

Another reason for the increase with time of radioactivity in the mono- and disaccharides may be their secondary formation from radioactive compounds which have already entered the phloem.

Counting of the chromatogram showed that with a 30-minute outflow a large part of the radioactive material is located above sucrose.

Mobility of the radioactive materials in the alcohol extract in the butanol – acetic acid – water mixture is shown in the following table:

Distance from the origin in cm	Radioactivity in counts/ min
0–1.5	40
1.5–4.0	20
4–7.0	0
7–9.0	0
Sucrose 9–12	20

TABLE 1

Appearance of Radioactive Carbon in Mono- and Disaccharides of Stems of 25- and 50-Day-Old Pumpkin Plants During Various Periods of Outflow

Group of substances	25-day-old plants				50-day-old plants			
	30 min		80 min		30 min		60 min	
	in counts/min	in %	in counts/min	in %	in counts/ min	in %	in counts/min	in %
Alcoholic extract	325	100	1300	100	1110	100	8300	100
Sucrose	46				246			
Glucose	9 } 64	20	500	38	27 } 300	28	3650	44
Fructose	9				27			

Thus, after a short-term outflow more than 70% of the radioactivity of the alcoholic extract is found in the relatively immobile (in B-A-W) fraction.

As we have previously shown [11], purification by passage through an ion exchange column only slightly reduces the radioactivity of the alcohol soluble substances in the stem, and therefore the compounds in which we are interested are not of an ionic nature. According to their position on the chromatogram, they closely correspond to slow moving ketose-containing oligosaccharides (Fig. 1).

On radioautograms the darkest spots coincide with the parts of the chromatogram corresponding to oligosaccharides (Fig. 2).

A three-hour hydrolysis with sulphuric acid (0.55N) at 100° of the radioactive eluate led to the formation of radioactive hexose containing the greater portion of the radioactivity of the original compound.

For a more detailed investigation these oligosaccharides were removed from a purified alcoholic extract by paper partition chromatography. After elution from the chromatogram and extraction from acetone they were placed in a vacuum desiccator over sulphuric acid. In this manner about 20 mg of material were obtained.

The material had no iodine reducing capacity. Determination of fructose according to Rowe's method showed that it comprised no more than 20% of the oligosaccharide. Judging from the mobility of these compounds on the chromatogram, there must be 4 or 5 hexose residues in them. The oligosaccharides were hydrolyzed with sulphuric acid (0.55N) at 100° for three hours. Separation of the products of hydrolysis in a mixture of butanol – pyridine – water (6: 4: 3) disclosed the presence of both flucose and galactose (Fig. 3, 2).

With a five-minute hydrolysis by sulphuric acid, fructose was completely split off from the oligosaccharide. Its removal uncovered an aldehyde group, as a result of which the remaining portion of the molecule could be reacted with aniline phthalate; the ability to react with urea was completely lost, in contrast with the original material which was detected by urea and which reacted very weakly with aniline phthalate (Fig. 3).

Hydrolysis by invertase also resulted in complete removal of fructose; in this case the remaining portion of the molecule also reacted with aniline phthalate (Fig. 3, 3). Glucose and galactose were formed in addition

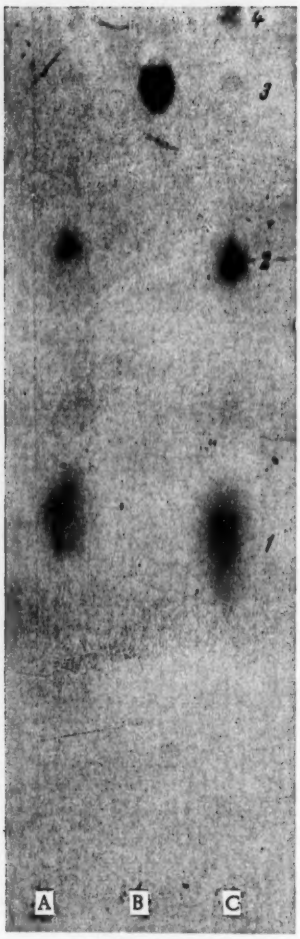

Fig. 1.

Fig. 2.

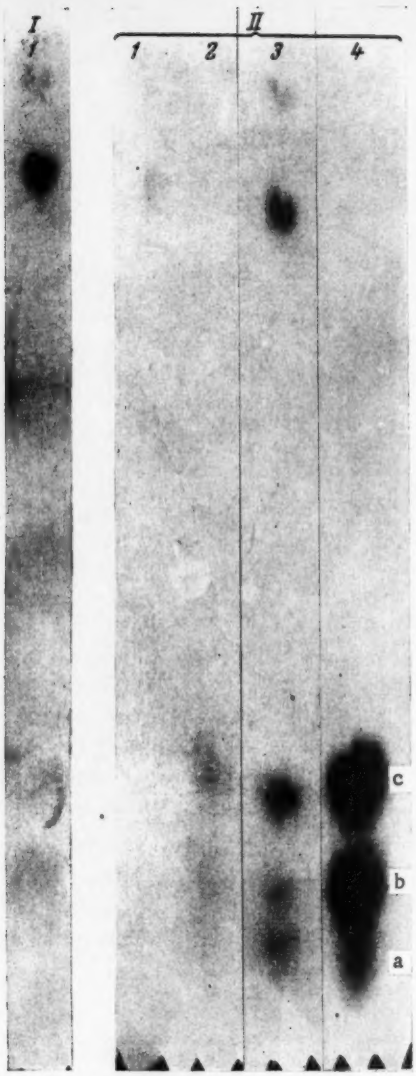

Fig. 3.

Fig. 1. Chromatogram of carbohydrates in an alcoholic extract from pumpkin stems.
Solvent system: butanol – acetic acid – water (4 : 1 : 5); sprayed with urea. 1) Fructose; 2) sucrose; 3) raffinose; 4) oligosaccharides. A and C) Extract from pumpkin stem; B) raffinose marker.

Fig. 2. Radioautograph of chromatogram of carbohydrates from the stem after a 30 minute outflow of assimilates.
Solvent system: butanol – acetic acid – water. 1) Sucrose; 2) raffinose; 3,4) oligosaccharides.

Fig. 3. Chromatogram of products of acid and enzymatic hydrolysis of oligosaccharides.
Solvent system: butanol – pyridine – water (6 : 4 : 3). I) Sprayed with urea; II) with aniline phthalate. I, 1 – Original oligosaccharide (sprayed with urea); II, 1 – original oligosaccharide weakly reacing with aniline phthalate; 2) hydrolysis with sulphuric acid for 3 hours; 3) hydrolysis with invertase containing a galactosidase contaminant; 4) markers. a) Fructose; b) glucose; c) galactose.

TABLE 2

Outflow and Conversion of Radioactive Assimilates in Stems of 25-Day-Old Pumpkin Plants (in counts/minute/g wet weight)

	Exptl. conditions	Radioactivity							
		stems		alcoholic extract	unextracted residue	oligosaccharides		sucrose	
		in counts/min	in %			in counts/min	in % of alcoholic extract	in counts/min	in % of alcoholic extract
1	Outflow 40 min	4225	100	4100	155	2280	58	685	17
	Outflow 40 min plus 150 min. after removal of leaf	2650		1430	1220	171	12	555	40
		1605	38	−2670	+1070	−2109		−130	
2	Outflow 40 min	3465	100	3280	185	1800	56	715	22
	Outflow 40 min plus 150 min after removal of leaf	1890		1110	780	84	9	264	28
		−1575	45	−2170	+595	−1716		−451	

to fructose during invertase hydrolysis. The appearance of galactose is apparently due to the presence in the invertase preparation of galactosidase activity.

Judging from the ease of removal of fructose, it would appear that it occupies a terminal position in the molecule and has the furanose form.

The chromatographic behavior of the molecule after removal of fructose is little different from that of the original substance (cf. Fig. 3, 1 and Fig. 3, 3).

A comparison of the properties of our substances with those of galactose-containing oligosaccharides described in the literature [12] enables us to define the nature of our compounds.

Their behavior in a butanol – pyridine – water mixture relative to galactose is the same as that of the tetrasaccharide stachyose and the pentasaccharide verbascose [12].

The behavior of the products of invertase hydrolysis is the same as that reported for manninotriose and verbascotetrose, which are obtained by hydrolysis of stachyose and verbascose with invertase. Finally, fructose makes up 20-25% of the total carbohydrate in these materials.

All this leads us to conclude that the oligosaccharides of the stem of pumpkin are stachyose and verbascose, formed by the union of two or three galactose residues to the glucose portion of the sucrose molecule (see scheme below):

$$\frac{\text{glucose } (1 \rightarrow 2) \text{ fructose}}{\text{sucrose}}$$

$$\frac{\text{galactose } (1 \rightarrow 6) \text{ glucose } (1 \rightarrow 2) \text{ fructose}}{\text{raffinose}}$$

$$\frac{\text{galactose } (1 \rightarrow 6) \text{ galactose } (1 \rightarrow 6) \text{ glucose } (1 \rightarrow 2) \text{ fructose}}{\text{stachyose}}$$

$$\frac{\text{galactose } (1 \rightarrow 6) \text{ galactose } (1 \rightarrow 6) \text{ galactose } (1 \rightarrow 6) \text{ glucose } (1 \rightarrow 2) \text{ fructose}}{\text{verbascose}}$$

TABLE 3

Radioactivity of a Portion of the Stem of a Dissected and an Intact Plant 150 Minutes after Removal of the Exposed Leaf

Expt. No.	Exptl. conditions	Radioactivity of the stem	Change in radioactivity in % of the original level
1	Plants cut up, stem sections kept 150 min in a damp chamber	3680	100
	Plants left intact, outflow for 150 min after removal of leaf	2300	
	Difference	−1380	38
2	Plants cut up, stem sections kept 150 min in a damp chamber	3640	100
	Plants left intact, outflow for 150 min after removal of leaf	2000	
	Difference	−1640	45

The lowest homologue of the galactose-containing substances of this series is isoraffinose.

The presence in pumpkin stems of a carbohydrate behaving like raffinose on a chromatogram (Fig. 1, 3) suggests the formation of oligosaccharides by successive additions of galactose residues to the sucrose molecule.

The high radioactivity of stachyose and verbascose coupled with their occurrence in small amounts indicates a rapid turnover, which may in turn be due to a rapid utilization in biosynthetic processes or to a constant flow from the stem into other parts of the plant.

The following experiment was performed in order to clarify the degree of participation of stachyose and verbascose in translocation and in secondary transformations.

Eight 25-day-old plants were exposed to radioactive carbon dioxide through one leaf for 20 minutes. After 40 minutes' outflow of the radioactive assimilates half of the plants were fixed for determination of radioactivity of the stems. The entire portion of the stem below the treated leaf was used for analyses.

In the remaining plants the radioactive leaves were removed, after which radioactive materials were allowed to flow into the stem and undergo conversions for a further 2 $^{1}/_{2}$ hours.

Changes in radioactivity of these substances occurring during this period give rise to the assumption of their participation in translocation and in secondary syntheses.

Results of two parallel experiments are presented in Table 2.

The data presented show that the total radioactivity of the stem 150 minutes after removal of the radioactive leaf is reduced to 38-45% of its original value. This reduction occurs in the alcohol soluble fraction. Accompanying this is an increase in the radioactivity of substances not extracted by the alcohol, which indicates the secondary character of their formation.

The decrease observed in total radioactivity of the stem is due in part to the flow of radioactive materials into other organs of the plant and to loss as carbon dioxide in respiration.

The following experiment was performed in order to assess the magnitude of loss of radioactive carbon by flow out of the stem.

Eight plants were exposed at the same time to radioactive carbon dioxide for 20 minutes, the exposure being given to one leaf. After a 40-minute outflow of radioactive assimilates the exposed leaf was removed, half the plants were left thus for 2 $^{1}/_{2}$ hours more, and the stems of the other half were divested of all leaves and cut into sections 4-5 cm in length which were then placed in a damp chamber and left for 2 $^{1}/_{2}$ hours.

A comparison of radioactivity of parts of the stem in these two groups yielded evidence of disposal of radioactive substances by outflow and by respiration. As in the previous experiment, portions of the stem below the exposed leave were utilized in analysis.

Results of two parallel experiments are presented in Table 3.

In the experiments summarized in Table 3, the two groups differed in the presence or absence of flow out of the stem of assimilates while the respiratory rate was relatively unchanged, so that the reduction in radioactivity is a measure of the extent of outflow in this case.

The radioactivity of stems of these plants, in which assimilates were permitted to move out of the stems for 150 minutes after removal of the exposed leaf, is reduced by 38-45% of the original value, which is the same as the radioactivity of the stem sections in which outflow is impossible. This compels the conclusion that the reduction in radioactivity of the stem after removal of the exposed leaf is mainly related to the outflow of assimilates into other parts of the plant.

The diminution of radioactive oligosaccharides in stems of plants whose exposed leaf has been removed comprises 80% of the decrease of alcohol soluble substances, and is 2-3 times greater than the increase in radioactivity in the unextracted residue. This leads to the conclusion that the reduction of radioactivity of oligosaccharides which was observed in our experiments is related not so much to their participation in secondary conversions as to carbohydrate translocation.

The radioactivity of sucrose also decreases in the course of the experiment, but to a smaller extent than oligosaccharide radioactivity, which apparently indicates that sucrose in involved in carbohydrate transport to a smaller extent in this plant.

In this connection the recently published paper of Zimmerman, who found a significant predominance of stachyose and verbascose over sucrose in the phloem exudate of white ash and American elm, should be mentioned [13]. According to his data, removal of the leaves of ash resulted in the reduction of the content of dry matter in the phloem exudate, with a concomitant decrease in stachyose. At the same time, an increase in the relative as well as the absolute amount of sucrose in the phloem exudate was found [14]. The utilization of stachyose in carbohydrate transport in representative of varios systematic groups: Oleaseae, Ulmaceae [13, 14] Cucurbitaceae (our experiments) shows that sucrose is not always the only transport form of carbohydrate. Evidently, in a number of plants it is replaced by oligosaccharides of the stachyose and verbascose type which are synthesized from it.

SUMMARY

The movement and transformation of organic substances in the stems of pumpkin plants, Cucurbita pepo, were studied with the aid of radioactive carbon.

It was found that after 30 minutes of flow of the radioactive assimilates from the leaves up to 90% of the radioactive substances in an alcohol extract from the stem were carbohydrates. Not more than 31% of these were mono-and disaccharides.

Most of the radioactivity is concentrated in alcohol-soluble oligosaccharides.

With increase of the duration of flow from the leaves the relative radioactivity of these substances decreases and the relative radioactivity of monosaccharides and sucrose increases. This seems to indicate secondary formation of these substances in the stem tissues.

A study of the chromatographic mobility and hexose components of the oligosaccharides permits one to identify them as stachyose and verbascose.

If the radioactive leaf is removed after a brief period of penetration of the assimilates in the stem, the amount of radioactive stachyose and verbascose is found to rapidly drop as a result of movement of these substances to other parts of the plant. The radioactivity of mono- and disaccharides was found to decrease to a smaller extent in this case.

It is my privilege to express by deep gratitude to Academician A. L. Kursanov for this continued interest in the work.

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THE SYNTHESIS OF CHLOROPHYLL IN THE DARK IN ANGIOSPERMS

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For a long time it was generally agreed that the final stage in chlorophyll biosynthesis took place in the vast majority of plants only in the light. Many of the lower plants and the gymnosperms were exceptions. Sachs had already observed the capacity of the letter to green up in complete darkness, it is true, although this was restricted to seedlings still dependent on endosperm.

In recent years, there have been individual observations indicating that at least some angiosperms are capable of forming small amounts of chorophyll in complete darkness.

R. Goodwin and O. Owens reported in 1947 that certain amounts of chlorophyll were found in Avena sativa seedlings grown in complete darkness [1]. They did not publish quantitative data, but from the published graph it may be concluded that the quantity of chlorophyll a which was formed in the dark was about 10% of that which was accumulated in the same seedlings after a three-hour exposture to light. No chlorophyll b was found in the etiolated seedlings, only chlorophyll a.

G. Röbbelen, investigating the formation of chlorophyll in normal plants of Arabidopsis thaliana and its x-ray mutants, noted that the normal plants formed chlorophyll both inlight and in complete darkness, while the mutants had lost the ability to synthesize chlorophyll under any conditions [2]. G. Röbbelen found, as had earlier workers, that only chlorophyll a is present in etiolated seedlings and in very small quantities, $5 \cdot 10^{-5}$ mg per gram fresh seedling weight, whereas the normal content of green plants is 0.9 mg. The quantity of chlorophyll formed in the dark was about 2% of the protochlorophyll of the sample.

In the light of these reports, the previously almost unnoticed experiments of A. Seybold and K. Egle, in the course of which it was found that in certain plants grown originally in the dark, then illuminated for a certain time and once more placed in the dark, the amount of chlorophyll in a number of cases not only did not decrease, but even increased quite markedly, assume a fresh importance [3]. It is true that these workers did not establish clear-cut patterns, and the chlorophyll content of plants transferred to the dark in many cases first declined, then once more rose, sometimes quite sharply. Perhaps this accounts for the fact that this study, of interest from many points of view, did not arouse comment at the time of its publication. Besides, the authors themselves did not attempt a theoretical interpretation of their results.

The study of the behavior in the dark of plants which have already accumulated a certain amount of chlorophyll is of great interest, especially because under these conditions the metabolism is more favorable than in etiolated plants. The enzyme systems involved in chlorophyll biosynthesis have evidently already been activated by light and may continue for a certain time to function even under conditions in which the plant is receiving no additional light energy. It is proper to note that an analogous situation is constantly encountered in nature in the alternation of day and night.

It is inadequate to study this process by the usual methods of a periodical determination of pigment content in living tissue. In previous years the studies of Roux and Husson [4, 5], Turchin and co-workers [6, 7], Shlyk and Godnev [8-12], Kutyurin [13] and others have established that the formation of chlorophyll is usually accompanied by the simultaneous breakdown of part of its molecules (a phenomenon called turnover), so that the pigment content is determined by the ratio of the rates of the two processes. It is therefore easier to make an accurate assess-

Fig. 1. Compound column for chromatographing pigments.

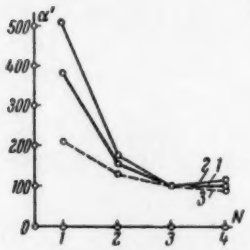

Fig. 3. Change in specific activities of chlorophylls a and b (α') in relation to the number of chromatographing (N) dark experiments). 1 - average values for chlorophyll a and chlorophyll b; 2 - average value for chlorophyll a; 3- average values for chlorophyll b.

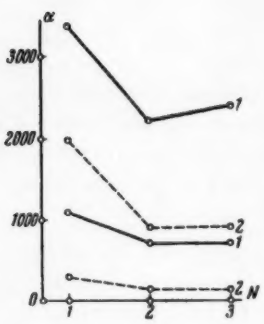

Fig. 2. Change in specific activities (a) of chlorophylls a and b in relation to the number of chromatographing (N). 1 - chlorophyll a; 2 - chlorphyll b.

ment of chlorophyll biosynthesis in green plants in the dark using the isotope method similar to that utilized in the study of turnover.

In view of the fact that in the solution of this problem it is not the quantitative assessment of chlorophyll biosynthesis in the dark which is of primary importance, but the adduction of evidence that it takes place at all, it was necessary to take special pains to obtain pigment preparations of the greatest purity for the measurement of radioactivity. In our previous studies it was shown [14] that ordinary chromatography of chlorophyll on a sucrose column does not guarantee complete purity of the samples obtained and that they should be further chromatographed on paper. In addition to our previous method of assessment of preparation purity by comparison of a chromatogram and its radioautograph, which is utilized in experiments described later, degree of purification was estimated by the constancy of the specific activity in a succession of chromatograms obtained in various ways.

The procedure in a typical experiment is as follows. Seeds of terrestrial plants were planted in a crock fitted with a glass column for the generation of carbon dioxide. When the plants were a week old and had formed green leaves, the vessel was hermetically sealed and placed in the dark after which labeled carbon dioxide gas was generated in the vessel. The plants were kept in complete darkness from 3 to 10 days at a temperature of 20-22° and then fixed by steam for two minutes.

In the study of aquatic plants, uninjured individuals which had grown under natural conditions were placed in a crock filled with water taken from the place the plants were collected. Subsequently sodium carbonate labeled with C^{14} was added to the water in complete darkness.

TABLE 1

Change in Specific Activities of Chlorophylls a and b during a Series of Chromatographings (in a dark experiment); (specific activity after the third chromatographing set at one hundred)

Plant	Exposure (in days)	Chlorophyll	No. of chromatographing			
			1	2	3	4
Ceratophyllum demersum L.	4	*a*	502	127	100	159
		b	147	70	100	138
Potamogeton perfoliatus L.	4	*a*	288	84	100	—
		b	65	75	100	101
Elodea canadensis L.	4	*a*	147	55	100	138
		b	505	91	100	41
Ceratophyllum demersum L.	10	*a*	1108	576	100	—
		b	133	197	100	87
Phaseolus multifloris L.	4	*a*	—	48	100	55
		b	—	218	100	84
Average for chlorophyll a			511	178	100	117
Average for chlorophyll b			213	130	100	90
Average for chlorophyll a and chlorophyll b			362	154	100	100.4

TABLE 2

Incorporation of C^{14} into Chlorophyll in the Dark by Various Plants

Plant	Expt. No.	Exposure (in days)	Specific activity, in counts per min per mg C	
			chlorophyll a	chlorophyll b
Ceratophyllum demersum L.	1	5	874	760
	2	4	73	78
	3	10	103	81
Elodea canadensis L.	4	5	254	264
	5	4	115	56
Potamogeton perfoliatus L.	6	4	104	148
Phaseolus multifloris L.	7	4	2650	367

The material fixed by the method described was ground in a mortar with a small amount of sand and calcium carbonate and was then extracted with acetone till all the pigment was removed. The acetone extract was filtered through a glass filter, transferred to a separatory funnel, and then the pigments were extracted into petroleum ether in the presence of an aqueous solution of sodium chloride. The water-acetone layer was drawn off, and the petroleum ether layer was washed once with water to completely remove the acetone and traces of water and then dried with sodium sulphate. The petroleum ether solution of pigments thus obtained was then chromatographed on a sucrose column of the type described previously [15].

The column consisted (Fig. 1) of a series of Allen tubes with open funnel-shaped ends and could be assembled and dismantled during chromatography. A pigment zone in a tube could then be further developed individually by a suitable solvent mixture. This method, then, was comparable to the method of successive chromatograms. The developed pigment zone was thus confined to a single tube and could be immediately eluted with carefully purified [16] ethyl ether; with the subsequent determination of radioactivity by a Geiger counter and of pigment concentration by a SF-4 spectrophotometer, the specific activity could be calculated.

As was pointed out earlier, column chromatography alone does not guarantee complete purification of a preparation. To assure this we therefore carried out further two-dimensional paper chromatography using solvent

systems suggested by Sapozhnikov and co-workers [17], but somewhat modified by us: first direction — benzene and petroleum ether (3 : 1), second direction — petroleum ether and ethyl alcohol (7 : 1). In almost all cases the specific activity was reduced after such a treatment, indicating insufficient purification on the column. It was still unclear, however, whether the chlorophyll could be considered to be completely free of radioactive contaminants even after such treatment. Therefore chlorophyll a and chlorophyll b were chromatographed a third time on paper in a solvent system suggested by Bauer [18]: first direction — special benzene ("rubber" benzene), petroleum ether, and acetone (10 : 2.5 : 2), second direction — special benzene, petroleum ether, acetone and methyl alcohol (10 : 2.5 : 1 : 0.25). The determination of specific activities of chlorophyll a and chlorophyll b, carried out as described above, showed that although even with a third chromatographing there is a certain reduction of activity, it is much smaller than between the first and second chromatograms and therefore it is extremely probable that in this stage the pigments have been completely purified.

In order to confirm the purification pattern observed, we performed a few control experiments in which we obtained chlorophyll labeled with C^{14} by the usual method of biosynthesis and turnover in the light. The chromatographing procedure was the same as described above. The curves of Fig. 2 showing the change in specific activities in relation to the purification stage strikingly illustrate the purification pattern. It is clearly evident that with the third chromatographing there is no decline in the specific activity in chlorophyll a or chlorophyll b.

Nevertheless, we chromatographed these compounds a fourth time in order to demonstrate still more clearly the purity of the pigment preparations obtained in the basic dark experiments; a new solvent system, suggested by Lind et al. [19] was used: first direction — petroleum ether, isopropyl alcohol (99 : 1), second direction — petroleum ether, chloroform (3 : 1).

The data obtained actually showed that after the third chromatographing complete purification of the pigments is attained, and subsequent chromatographing does not result in any change in the specific activity.

The results of determinations of specific activities of each of the chlorophyll components during purification are summarized in Table 1, the raw data being expressed relative to the value obtained after the third chromatographing, which is set at 100. In spite of the disparity among individual values, which was due to varying degrees of purification (first and second chromatograms) or to small quantities of pigment (particularly at the time of the fourth chromatographing) with the resultant difficulties of direct determination, the average values were in agreement in indicating an extremely high level of purity. This is especially clear in Fig. 3, which shows the change which occurs during a series of chromatographings, in average values of the specific activities of chlorophylls a and b obtained in a dark experiment.

Since the preparations carefully purified in this manner exhibit considerable activity, as shown in Table 2, there is no reason to doubt the incorporation of C^{14} from carbon dioxide or carbonate into the chlorophyll molecule by plants in complete darkness. In other words, the ability of the angiosperms studied to synthesize chlorophyll in the dark may be regarded as a proven fact.

As was pointed out above, the purpose of the work at this stage was to demonstrate that higher angiosperms are able to synthesize chlorophyll in the dark, and not to study in detail the character of such a process. From this point of view, it appears to us that the work described has led to definite positive results. Any conclusions as to the dynamics of the process, its mechanism, or the relation of the biosynthesis of chlorophylls a and b are, however, still premature.

Therefore, there is in higher plants, in addition to a photochemical system which mediates normal synthesis of chlorophyll, an enzyme system acting in an analogous manner in the dark. It had already been shown in 1952 by Godnev and Terent'ev [20] that by infiltrating etiolated leaves of corn with sap obtained under high pressure from fir seedlings (which are able to form chlorophyll in the dark) it is possible to induce conversion of a certain portion of the protochlorophyll into chlorophyll in the absence of light. This was later confirmed by the experiments of Röbbelen [2] on Arabidopsis thaliana mutants. Now we have shown that not only may the enzyme system involved be introduced into angiospermous plants from external sources, but that it is also present in these plants. It is possible that it is activated by light in the case of plants which have already accumulated chlorophyll; however it functions to some extent in etiolated seedlings as well.

In addition to the data considered above, it may be indicated that in our laboratory T. N. Godnev and N. K. Akulovish have found by the spectral method that very small quantities of chlorophyll appear during etiolation in certain other angiosperms. At present it is difficult to decide whether there is a system mediating dark synthesis which supplements that acting in the light, or whether dark synthesis is mediated by a system which is a component part of the light system.

It may be thought that in the evolutionary process there was a degeneration of this enzyme system in the overwhelming majority of higher plants such that it retained only a slight activity in the absence of illumination; however, at least in certain representatives of the angiosperms, this reduction was incomplete, and a rudimentary enzymic apparatus for the protochlorophyll→chlorophyll reaction was partially preserved. It is interesting to recall that according to the data of Smith and Beniter [21] the photochemical conversion of protochlorophyll into chlorophyll also shows clear signs of being a catalytic process, since it possesses a perceptible temperature coefficient, although this is much smaller than with ordinary enzymatic reactions. The process occurs at especially low temperatures and is described by an equation of the second degree.

It should be noted that as yet it has not been determined to what degree dark synthesis is concerned with the synthesis of the component parts of the chlorophyll molecule — phorbin and phytol. Preliminary experiments involving the cleavage of chlorophyll labeled in the dark have indicated a small activity in the phorbin as well as the phytol portion of the molecule; these experiments require elaboration, however.

SUMMARY

It is shown that not only lower and gymnospermous plants but also some angiospermous plants are capable of forming perceptible amounts of chlorophylls a and b in the dark although the amounts of chlorophyll a and b formed are much smaller than in light and depend on the nature and age of the plants.

This could be inferred from the radioactivity detected in preparations of chlorophyll a and b of terrestrial angiospermous plants placed in a $C^{14}O^2$ atmosphere and of aquatic angiospermous plants immersed in a solution containing $C^{14}O_3$ and $HC^{14}O_3$ anions. The radioactivity was found to depend on the nature and age of the plants.

Judging from the radioactivity of elaborately (threefold) chromatographically purified preparations the amounts of chlorophyll formed in green plants were much greater in some cases than those observed in some etiolated angiospermous plants. (Goodwin, Röbbelen).

It is highly probable that the enzyme apparatus which was simplified during the process of evolution and which is well developed in lower and gymnospermous plants and transforms protochlorophyll into chlorophyll, is also partially retained to various degrees in some angiospermous plants.

Light energy stimulates this enzyme system. This is consistent with the fact that brief illumination with weak red light and to a less extent with violet light stimulates the transformation of protochlorophyll into chlorophyll and the subsequent formation of chlorophyll.

To all appearence this is related to the formation of enzyme stimulating substances under the influence of red light and, to a much less extent, under the influence of violet light.

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INFLUENCE OF WILTING ON REDOX CONDITIONS IN PLANTS

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The rate of oxidative-reductive processes is of basic significance in the life of plants since the overall metabolism is bound up with these processes.

Up till the present time little work on oxidative-reductive (redox) conditions in plants and their relation to various internal and external factors has been published.

With the introduction of a method of quantitative evaluation of the redox potential, a number of workers showed that the Eh and rH vary widely in different plants and in different organs of one and the same plant [1, 2]: redox conditions are subject to internal and external factors. Thus, it was shown that during sprouting of reserve tissues the redox level is altered.

In the opinion of V. N. Lyubimenko, the level of oxidative-reductive activity in plant cells is of basic importance in chlorophyll formation. This point of view was later confirmed by the studies of Gortikova and Sapozhnikov [3]. They showed that for chlorophyll formation (specifically for chlorophyllogen formation) a definite pH is necessary. Okanenko and Ostrovskaya [4] determined the oxidative-reductive properties of tissues of kok-saghyz* and sugar beet by means of their interaction with positively and negatively charged electrodes. It turned out that in beet the maximum potential was higher in the roots than in the leaves. In kok-saghyz on the contrary, the roots are distinguished by a high reductive capacity. The authors attribute this to the formation in the roots of kok-saghyz of reduced compounds which are early precursors of rubber.

Geller [5], in a study devoted to the problem of the influence of oxygen and other oxidants on the redox potential of sugar beet tissues, came to the conclusion that the redox status of a plant is the result of the interaction of a number of factors, among which the redox potential of the soil is of importance.

In view of the dependence of the redox potential Eh on internal and external conditions, we carried out investigations of the effect of water deficiency on the redox status of leaf cells.

METHODS

As experimental material we used representatives of the grains (millet), legumes (lupine), vegetable plants (cucumbers, kidney beans), commercial crop plants (poppy, Turkish tobacco) and horticultural plants (sweet tobacco). Leaves of an intermediate age (fifth-sixth nodes) were used. To obtain a correct view of the shift of physiological-biochemical processes elicited by water deficiency, it was considered extremely important to study the influence of wilting in intact leaves and in leaves removed from the plant. For experiments with isolated leaves, plants were grown outside in the ground, and for experiments with intact wilted plants, they were grown in basins.

In the study of isolated leaves, 16-20 leaves were removed from 8-10 plants of each species at a given time. Half of the leaves were placed petiole downward in water, and the other half were allowed to wilt, being placed on a table under illumination identical with that of the control leaves.

Analyses of control and wilted leaves were performed 9 hours after the beginning of the experiment. Experiments were replicated three times.

*Taraxacum kok-saghz.

TABLE 1

The Effect of Wilting on Eh, pH, rH and Reducing Strength of Leaf Tissues (leaves detached from plants)

Plant	Year	Values			Reducing strength	
		Eh, pH, rH	control	experimental	control	experimental
Opium poppy	1947	Eh	0.3497	0.3857	8	5
		pH	5.9	5.4		
		rH	23.9	24.1		
Sweet tobacco	1947	Eh	0.3050	0.3443	10	9
		pH	6.0	5.4		
		rH	22.6	22.7		
Nerosimy cucumber	1948	Eh	0.1700	0.1890	12	10
		pH	8.5	8.3		
		rH	22.8	23.2		
Turkish tobacco	1948	Eh	0.3146	0.3361	10	7
		pH	6.5	5.9		
		rH	22.8	23.3		
Narrow-leaved lupine	1948	Eh	0.2910	0.3073	10	7
		pH	6.2	5.7		
		rH	22.4	21.9		

Experiments with wilting of intact plants were carried out as follows. Plants of each species were grown in 30 basins, with those in 15 basins serving as controls. As a certain stage of development was attained (flowering, heading, etc.), watering of experimental plants was curtailed and plants were deprived of water for from 4 to 8 days. During this time, in plants which had not been watered, the lower leaves lost turgor completely, the middle leaves became strongly wilted, and the upper leaves retained the appearance of turgor, without however being fully turgescent. Leaves of control plants were completely turgescent. Subsequently leaves of an intermediate age (fifth and sixth nodes) were removed from part of the plants (from six to seven) in both the control and experimental groups and Eh, pH, and reducing strength were determined.

Eh and pH were determined electrometrically with platinum electrodes (N. P. Krasinskii's modification). Reducing strength was determined by the method of Tadokoro.

The work was performed in the Sverdlovskaya greenhouse of Gor'kii in 1947-1948. Analyses were carried out in the laboratory of plant physiology and biochemistry of the Gor'kii state university.

EXPERIMENTAL PART

In Tables 1 and 2 are presented figures on the changes in redox status of leaf cells associated with leaf wilting.

In Table 1 it can be seen that in wilted leaves detached from plants the redox potential was increased: in sweet tobacco by 0.036 v, in poppy by 0.036 v, in Turkish tobacco by 0.018 v, in lupine by 0.016 v and in cucumbers by 0.019 v.

In leaves of plants grown in basins, changes in Eh during wilting which were analogous to those occurring in detached leaves were observed.

Analyses of leaves performed 4-8 days after cessation of watering, when wilting of the lower and middle leaves was clearly evident, showed that the redox potential was significantly increased in the experimental plants.

The greatest change in the redox potential was found in millet leaves. Thus, 6 days after the cessation of watering the redox potential of the cellular contents had increased by 0.078 v in 1947 and by 0.032 v in 1948. The increase was less marked in cucumbers and kidney bean.

TABLE 2

The Effect of Wilting on Eh, pH, rH and Reducing Strength of Leaf Tissues (leaves not detached from plants)

Plant	Year	Eh, pH, rH	Values: control	Values: experimental	Reducing strength: control	Reducing strength: experimental
Red kidney bean	1947	Eh	0.3555	0.3830	6	3
		pH	5.8	5.7		
		rH	23.9	24.6		
	1948	Eh	0.3323	0.3710	8	4
		pH	6.1	5.9		
		rH	23.6	24.5		
Millet	1947	Eh	0.3479	0.4260	9	6
		pH	6.0	5.3		
		rH	24.0	25.3		
	1948	Eh	0.3160	0.3486	7	6
		pH	5.8	5.6		
		rH	22.4	23.1		
Nerosimy cucumber	1948	Eh	0.2041	0.2134	6	4
		pH	8.5	8.3		
		rH	24.0	24.1		

On the basis of the data presented in Table 2 it is possible to calculate that during a 4-8 day period of water deprivation the redox potential in leaf tissues was increased by 0.038-0.078 v in millet, by 0.027-0.039 v in kidney bean, and by 0.018 v in cucumbers.

Comparing the data for detached and undetached leaves, it may be stated that Eh does not increase to a lesser extent in the latter case than in the former case. In the experimental (wilted) leaves there was associated with the increased redox potential an increased hydrogen ion concentration of the intracellular material.

The depressed respiration of the wilted leaves undoubtedly contributed to the accumulation or organic acids and the concomitant rise in acidity, i. e. lowering of pH.

Analysis of the data of Table 1 shows that in 1947 there was a highly significant decrease in pH of detached leaves of sweet tobacco during wilting (from 6.0 to 5.4) as well as in leaves of opium poppy (from 5.9 to 5.4). In 1948, the most marked reduction of pH was observed in leaves of Turkish tobacco (from 6.5 to 5.9) and of lupine (from 6.2 to 5.7). It should be noted that in lupine the pH determinations were not repeated: the figures are therefore only preliminary. The pH of cucumber leaves was very high, about 8.5. Similar pH values for cucumber were obtained in the studies of Krasinskii, Kon'kova and Nikol'ska. The decline in pH of cucumber leaves associated with wilting was smaller than in the other plants.

As Table 2 shows, the greatest change in pH of undetached leaves associated with wilting occurred in millet (from 6.0 to 5.3). In kidney bean leaves the decrease in pH was insignificant (from 5.8 to 5.7). In the 1948 experiments there was also an increase in the acidity, i.e., a reduction of pH, of cellular contents associated with wilting. For example, the pH fell from 5.8 to 5.6 in millet leaves, from 6.1 to 5.9 in kidney bean leaves, and from 8.5 to 8.3 in cucumber leaves. The work of Maksimov [6], Alekseev [7], Genkel' [8] and others has established that the death of plants in drought conditions occurs primarily as a result of a change in the condition of biocolloids. Maksimov [6] showed that one of the basic causes of the disruption of physiological processes attendant on wilting is a disturbance of the normal condition of the protoplasmic colloids. This disturbance is manifested especially clearly in a change in the osmotic properties of the protoplasm as well as in changes in permeability and viscosity.

One of the main factors involved in changes in biocolloid status during wilting is evidently the increasing acidity of the cellular contents.

With an increase in acidity the biocolloids approach the isoelectric condition in which, as is known, proteins, devoid of electrical charge, become the least stable [9, 10]. At the same time the biocolloids lose another stability factor — the layer of water surrounding the colloidal particles, i.e. the colloidal hydrophily

decreases, resulting first in coacervation and finally in true coagulation with a marked loss of biocolloidal stability attendant on water deprivation.

It should be pointed out that the increased acidity of the contents of leaf cells which accompanys wilting should act unfavorably by changing the condition of the biocolloids.

Wilting of leaves detached from plants induced changes in the aerobic index rH. In individual plants the rH fluctuated in a random manner from day to day. For example, opium poppy in two cases showed an increase in rH of 0.21-1.0 units, and in another case — a decrease of 0.6 units (see Table 1). Sweet tobacco in two cases showed a decrease of 0.70-0.6 units and in another case an increase of 1.4 units. In Turkish tobacco wilting induced a decrease of 0.5 units, based on three determinations. In lupine wilting induced a decrease of 0.5 units. In cucumbers, in 1948, there was an increase of 0.2-0.7 units associated with wilting. With leaves not detached from the plants, the following changes in rH as a result of wilting were noted. In millet, in 1947, there was an increase of 1.3 units in one case, and in another case an increase of 1.5 units. In the 1948 experiments there was an increase of 0.7 units. In kidney bean an increase of 0.4-1.0 units was observed in 1947 and an increase of 0.9 units in 1948. In cucumbers, in 1948, the rH remained unchanged in wilted leaves.

As the figures of Tables 1 and 2 show, there was a certain difference in the nature of the changes in rH associated with the wilting of detached as compared with undetached leaves. Wilting was more pronounced in detached leaves, which led to a greater reduction in pH than in undetached leaves. This is obviously due to the absence of an outflow of metabolic products into the stem axis with a resultant reduction of rH is many cases.

In the cells of leaves which had wilted on the plants the rH was increased to a greater or lesser extent, and this is certainly of interest.

The data obtained by us on changes in the redox status of leaves during wilting should be compared with results obtained by Mothes [11].

In his investigations devoted to the transformations of nitrogenous compounds Mothes [11] asserted that during leaf wilting a reduction in the oxidation potential occurs. He based his assertion, however, on theoretical considerations, and did not determine either the oxidation potential or the intensity of oxidative-reductive processes. His experiments did not embrace the determination of such important indicators of the intensity of oxidative-reductive processes in tissues as the redox potential, the hydrogen ion concentration, the aerobic index, the reducing strength or the dehydrogenase activity. A reduction of the oxidation potential should occur during wilting as a result of stomate closure and a decrease in the partial pressure.

The oxidation potential and the redox potential are two distinct matters, however.

Our experimental data indicate that during wilting of detached or undetached leaves the redox potential increases.

While Mothes asserts that with a loss of water from the leaves reductive processes begin to predominate, our determinations of Eh and also rH in undetached leaves showed that there is a shift in the direction of oxidative processes with wilting.

The decrease in the aerobic index rH in leaves detached from the plant is no criterion, since it is induced under these artifical conditions by a decrease in pH, of which we have already spoken. The decrease in reducing strength, which we determined by Tadokoro's method, confirms our assertion that oxidative processes become relatively more active during wilting.

Reducing strength is a measure of the reductive capacity of cells and can be thought of, in one sense, as being the converse of redox potential [12].

In the work of Krasinskii and Pryakhina it was shown that with a reduction of light intensity the redox potential rises and the reducing strength falls, i. e. these indices are altered in opposite directions.

It is possible, however, that the indicated relation between the redox potential and the reducing strength is not always expressed, since the redox potential is determined by the status of the redox system, and reducing strength by dehydrogenase activity.

Upon inspection of Tables 1 and 2, showing the influence of wilting on the reducing strength, it may be seen that in the course of a 9-hour wilting period there is a decrease in the reducing strength of the cells of detached leaves as compared with control leaves. The greatest decrease occurred in leaves of opium poppy, Turkish tobacco and lupine (3 units on the average).

In leaves left on the plants the reducing strength also decreased during wilting. It is evident from Table 2 that the sharpest decline occured in leaves of kidney bean (3-4 units), and also in leaves of millet in the 1947 experiments (3 units).

This decrease in reducing strength during wilting cannot be attributed to the increase of acidity of the cellular contents (which would lead to inactivation of dehydrogenases). In leaves not detached from plants wilting induced only a slight reduction of pH while the reducing strength was decreased markedly, if not to a greater extent than in detached leaves, certainly not less. Thus, consideration of our data on the redox status of turgescent and wilted leaves shows that they are at variance with the conclusion of Mothes that there is a shift in the direction of reductive processes associated with wilting.

SUMMARY

Investigations on the influence of wilting on the redox conditions in plants permit one to draw the following conclusions:

In contrast to the opinion of Mothes that wilting of leaves, in decreasing the oxidation potential, should enhance reducing processes, we have found that wilting of leaves not only does not change the biochemical processes towards a preponderence of reduction processes but, judging by the value of Eh (and also rH) it has the reverse effect, shifting the redox conditions in the leaf tissues to more marked oxidation conditions.

Simultaneously with increase of the redox potential in wilting leaves the contents of the cells became more acidic; this is apparently a result of impeded gaseous exchange which entailed only partial oxidation of energy-producing matter.

It has been established that wilting of leaves causes an increase in the rH of the cell contents which signifies a shift of the biochemical processes towards oxidation conditions. This is confirmed by a decrease in the value of the reducing power of wilting leaves in all experiments as compared with the reducing power in control leaves.

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* In Russian

CHANGE IN THE NUCLEIC ACID CONTENT IN RELATION TO THE GROWTH OF BUDS AND YOUNG SHOOTS IN TEA

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A peculiarity of tea cultivation, in contrast to the cultivation of many commercial crops, consists in the fact that the final product is the green leaf. The accumulation of green leaf tissue, determining the yield, is related to growth processes.

The biology of shoot formation in tea in Georgia has been thoroughly studied by Bakhtadze [1].

Our previous studies [2-4] of the growth of buds and shoots in tea, which were carried out under the subtropical conditions of Azerbaidzhan, showed that from the first favorable spring days to the winter chillings growth did not cease. It was shown in the same studies that during a dry summer in Azerbaidzhan, tea undergoes a prolonged period of enforced dormancy.

To obtain a deeper insight into growth processes in tea we decided to carry out careful observations of the nucleic acid metabolism of the growing points of the buds in relation to bud growth.

It has been established that nucleic acid metabolism is one of the basic elements in growth [5-13].

In our investigations of 1955 we studied the dynamics of nucleic acid in the growing points, in addition to making the usual observations.

Nucleic acid determinations were made in axillary and terminal buds. After removal of the buds they were fixed in Carnoy's solution (alcohol – chloroform – acetic acid in the proportion 6 : 3 : 1); sections were then washed in the same solution and placed in well purified acetone, where they were allowed to remain for 18-24 hours in order to remove tannin (as is known, tannin, which occurs in tea buds in large quantities, interferes with the nucleic acid reaction). Subsequently the sections, free of tannin, were washed and their content of ribonucleic acid (RNA) and desoxyribonucleic acid (DNA) determined.

RNA determination was performed according to Brasha, the sections being stained with azure-eosin according to Kedrovskii [11], DNA was determined by the Feulgen method [14].

The data presented in Table 1, describing the over-all growth of buds of tertiary rank, show changes in nucleic acid content of the growing points of the buds.

The accumulation of nucleic acids in tissues of unirrigated plants varied only slightly, but the values for buds of irrigated plants varied markedly. The determinations made on July 6 and 11 showed that there were buds rich in nucleic acids as well as buds with little or no nucleic acid.

Careful consideration of the data obtained led us to the supposition that in unirrigated plants, as a result of water deficiency, the buds underwent a complete transition to an enforced dormancy and ceased growth, including internal growth. Associated with this was a marked reduction in nucleic acid content.

It should be noted that this sharp reduction was characteristic of the RNA fraction. We found DNA to be present in all cases in unirrigated plants, albeit in smaller quantity than in irrigated plants.

TABLE 1

The Accumulation of Nucleic Acids in Tertiary Buds of Tea in Relation to Their Growth

Treatment	6.VII				11.VII				21.VII				1.VIII				12.VIII			
	Bud length in mm	No. of leaf primordia	RNA	DNA	Bud length in mm	No. of leaf primordia	RNA	DNA	Bud length in mm	No. of leaf primordia	RNA	DNA	Bud length in mm	No. of leaf primordia	RNA	DNA	Bud length in mm	No. of leaf primordia	RNA	DNA
Without irrigation	1.9	4.0	+	+	2.1	4.0	−	+	2.0	4.0	−	+	1.9	4.2	−	+	2.0	4.2	−	+
			++	++			+	++			−	++			++	++	Shoot			
Irrigation	2.0	4.0	+	+	2.4	4.6	−	+	3.4	6.2	−	+	10.6	8.3	+	+			+	+
			++	+++			+++	+++			+++	+++			+++	+++			++	+++

RNA content in irrigated plants varied, as did that of DNA, though to a lesser extent. Buds identical as to external characters contained in one case abundant amounts of RNA, and in another case very small amounts.

Subsequently, when some of the buds had entered a period of visible growth, the determination of nucleic acids in their growing points showed a high content of both RNA and DNA. Buds giving a weak RNA reaction had ceased growth and become dormant. Buds of irrigated plants which had commenced visible growth became actively growing shoots in 12 days (See Table 1).

Determining the nucleic acid content in the growing points of the terminal buds of these shoots, we are confronted with a paradox. Some of these buds, all of which were actively growing, showed a low content and some a high content.

We subsequently followed the growth of quaternary buds. These buds are laid down in the axil of the third leaf primordium of a tertiary bud. We observed these buds from the very beginning of their formation. Longitudinal sections of the tertiary buds were examined under a microscope and those which passed through the axillary buds were kept for further determination of nucleic acid content. In some cases these buds were measured and the number of lead primordia determined.

Study of the early phase of growth and development of quaternary buds presented decided difficulties. It was necessary to section many tertiary buds in order to obtain sections through the axillary buds of the third leaf primordium.

Determination of DNA and RNA content indicated that in all cases these substances are present in significant quantities in the beginning stages of bud development.

The first observations of the formation of quaternary buds were made on July 28. At this time there was no visible indication of a bud in the axil of the third leaf primordium of the tertiary bud, but the site at which the bud would be laid down was densely stained by acid fuchsin and the protoplasm of the cells of this area was distinguished by a high degree of basophilia. This picture was observed in the majority of cases. But cases were encountered in which the picture was different: there was no marked staining by acid fuchsin and no high degree of basophilia. As a rule, the growing points of the tertiary buds are distinguished by low nucleic acid content in such cases. In the majority of cases the region in which a quaternary bud will be laid down is characterized by a high level of nucleic acid.

Longitudinal sections of tertiary buds made on August 4 provided visible evidence of the existence of the quaternary bud. At this time a single leaf primordium was present in some cases. These buds were rich in DNA, and their growing points showed a high degree of basophilia.

TABLE 2

Nucleic Acid Content in Terminal and Branch Buds of Shoots of Various Ages, Aug. 10

Age of Shoots	RNA		DNA	
	terminal buds	axillary buds	terminal buds	axillary buds
One leaf	+	−	+ +	+ +
	+ + +	+ + +	+ + +	+ + +
Two leaves	+	−	+ +	+ +
	+ + +	+ + +	+ + +	+ + +
Three leaves	−	−	+ +	+
	+ + +	+ +	+ + +	+ + +

Note. The experiment was performed on buds taken from irrigated tea plants.

Our subsequent observations were made on buds located in the axis of the third leaf primordium, which was transformed into a scale leaf of a tertiary shoot.

At the beginning of the growth and development of quaternary buds a high level of RNA and DNA was found. The number of buds low in nucleic acids was small. As the growth of the tertiary bud and its conversion into a shoot continued the nucleic acid content of the growing points of the quaternary buds declined. By August 19 buds were encountered in which basophilia of the protoplasm of cells in the growing points was completely absent.

After the removal on August 22 of tissue containing the terminal buds from the tertiary shoots, the quaternary axillary buds took the place of the terminal buds, and on August 26 the amount of nucleic acids in the growing points of these buds had increased perceptibly. Once more these were found to be variable with respect to basophilia in their growing points and nucleic acid content; however, a significant number contained large amounts of nucleic acid. At the end of September many of these buds had grown into shoots,in the growing points of which there was a comparatively high concentration of both RNA and DNA

Observations of the growth of quaternary buds and changes in nucleic acid content of their growing points showed that even before the beginning of the morphological formation of these buds,there is a high basophilia and an intense staining reaction for nucleic acids in the areas at which the buds will appear. From the time of formation to the end of September some of these buds exhibited a rapid accumulation of nucleic acid in their growing points. In the process of individual development of the quaternary buds periods of lowered basophilia were observed. These periods corresponded with the period of active visible growth of the tertiary shoots on which the quaternary buds are located. Evidently the energy requirement and the growth of the terminal bud were reflected in processes taking place in the quaternary axillary buds. Upon removal of the terminal bud, which occurs in practice during harvest of the leaf crop, the quaternary axillary bud becomes terminal and the subsequent flow of nutrient supplies is toward this bud, which thus enters into an active growth period. During this period a high basophilia and an intense nucleic acid reaction in the cells of the growing points was noted.

In addition to the actively growing buds there were buds which had become dormant. In spite of the favorable conditions these buds had not become active at the end of the growing period. Such buds were distinguished by a low nucleic acid content in the growing points, and in the majority of cases RNA was completely absent. Buds of this type were encountered in the tertiary series as well, as was pointed out above. In spite of this, however, we found a systematic growth and development of buds and shoots of the tertiary and quaternary series from the beginning of the observation period to the end of September. At the same time, the accumulation in the growing points of these buds of nucleic acids was established.

We were further interested in whether there is a difference between the terminal bud and the axillary buds of normally growing shoots with respect to nucleic acid content. To determine this, three groups of young normally growing shoots were selected. The first group comprised shoots which had just begun visible growth. In such shoots there was one normal leaf in addition to a scale leaf.

In the second group were shoots with two normal leaves, and in the third, shoots with three normal leaves. In each case 20-22 buds were taken for study.

Results of a determination of nucleic acid content in the growing points of these buds are presented in Table 2. An axillary bud of the lowest leaf was taken for study.

The data in Table 2 show that the growing point of the terminal bud of a one-leaved shoot is rich in nucleic acids and the that shoot is in an actively growing condition. Certain axillary buds of this shoot, being

in the stage of internal growth, are also characterized by nucleic acids in the growing points. In this stage the RNA present mediates the growth and differentiation of the axillary buds. Other axillary buds, however, are in a dormant condition; their RNA content is insignificant although the DNA level is normal.

The terminal bud of two-leaved shoots also contains a sufficient quantity of RNA. This bud is in an actively growing condition. A significant amount of RNA in the growing points of many axillary buds was also detected in this case. These buds, with a prolonged period of internal growth, are differentiated and have an increased number of leaf primordia.

Three-leaved shoots, being of advanced age, have varying contents of nucleic acids. In some cases shoots are encountered whose terminal buds are richly supplied with RNA. At the same time there are shoots with a low content of RNA in the growing points. It is probable that these buds are at the end of their period of internal growth and have completed the process of differentiation. Ordinarily such buds subsequently, in the absence of favorable conditions, become dormant. This experiment shows that in addition to the growth of the terminal bud on a young shoot there is, up to a definite time, an internal growth and differentiation of axillary buds.

Observations of the accumulation and alterations in content of RNA in the growing points of buds of tea showed that this physiologically active substance plays an important role in the growth of young shoots and in the differentiation of growing points during internal growth. At the same time our experiments show that synthesis and accumulation of nucleic acids in plant tissues depends wholly on the external conditions of growth of the tea plant.

The most important environmental factor in the dry conditions of a summer in Lenkoran' is soil moisture. It has been shown that irrigation, improving the water status of the tea plant, creates favorable conditions for nucleic acid synthesis which in turn activates the growth processes of the plant.

I extend deep thanks to Professor P. A. Genkel' for valuable advice and discussion during the formulation and execution of this study.

SUMMARY

A prolonged period of forced dormancy in the tea plant was observed under conditions of a dry summer in Lenkoran'. During this period the nucleic acid content showed a marked decrease in the growing points of terminal as well as of axillary buds.

Irrigation of the tea plant leads to conditions under which the tea plant is devoid of dormancy throughout the whole vegetative period. In most cases the growing organs of watered tea plants have large amounts of nucleic acids. In some cases the content of nucleic acids in certain organs decreases as that particular organ changes to the dormant state.

The nucleic acid content (mainly RNA) in the growing points may indicate dormancy or active growth of buds and shoots in the tea plant.

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EFFECT OF SUCCINIC AND FUMARIC ACID TREATMENT OF SEEDS ON CERTAIN PHYSIOLOGICAL PROCESSES IN PLANTS

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In recent years the science of biogenic stimulators, which have found wide application in medical and veterinary practice [2], has been successfully developed [1].

With respect to their chemical composition biogenic stimulators comprise a diverse group of various organic substances such as, for example, amino acids and their oxidatively deaminated derivatives (dicarboxylic acids and oxyacids of the fatty acid series, unsaturated, aromatic and other acids).

The intimate site of action of biogenic stimulators in the metabolic process is as yet unknown. It is probable that they exert a deformational stress on enzyme proteins, thus bringing about an increase in their level of activity [3]. That this may be so is indicated by the fact that in plants which were subjected to a presowing treatment with biogenic stimulators the activity and efficiency of enzymes was, as a rule, higher than in plants which had not been so treated [3-5]. A presowing treatment of seeds with biogenic stimulators obtained from cold-stored and autoclaved plant tissues exerts a stimulating effect on germination of corn, on growth, development and yield of cotton, on growth, development and biochemical properties of peas, beans and kidney beans [11] and on yield of barley, oats, and spring wheat [4-8].

On the assumption that the principal components of biogenic stimulators are dicarboxylic acids and other products of protein breakdown, the action of these substances on various plants was tested. It was found that dicarboxylic and other acids in small concentrations exert a positive effect on the rate of seed germination, on the growth of roots and aerial organs, and on the size and quality of yield [9-13].

We have attempted to study the effect of presowing stimulation of seeds by dicarboxylic acid solutions on the content of water and unsaponified chlorophyll and on the catalase activity of leaves of Lyutestsens 62 spring wheat as well as on the chlorophyll content of the leaves of Odessa 10 corn.

Seeds of Lyutestsens 62 wheat and Odessa 10 corn were soaked for a day in 0.0002M solutions of fumaric and succinic acids prior to sowing. 100 seeds were soaked in 35 ml of solution. The treated seeds were sown on an experimental plot of the Melitopol' Pedagogical Institute. Seeds soaked in distilled water and dry seeds served as controls.

Water Content of the Leaves

There is an abundance of data showing that water content of leaves varies in accordance with temperature, irrigation conditions, plant ontogenesis and other factors [14, 15]. We determined under field conditions the water content of leaves of Lyutestsens 62 wheat throughout the growing period.

Up until the shooting stage water content was determined without distinction as to the nodal position of the leaves; in succeeding stages, however, strict attention was given to this factor. In all cases samples were taken for analysis at one and the same time — between 7 and 8 o'clock in the morning — weighed, and dried to a constant weight at 100-105°C. Experiments were replicated three times. Yellow and withered leaves were not used. The data obtained are assembled in Table 1.

TABLE 1

Water Content of Leaf Blades of Lyutestsens 62 Wheat in Relation to Presowing Stimulation and Developmental Stage (in % of absolute dry wt; modes numbered upwards)

Stage of development	Node number	Date of determination	Control – dry seeds	Control – seeds soaked in water	Seeds soaked in succinic acid	Seeds soaked in fumaric acid
First leaf		20.IV	81.09	81.01	80.83	80.98
Third leaf		26.IV	82.37	82.23	84.16	84.25
Tillering		5.V	79.80	79.96	83.20	83.12
Shooting	4	26.V	67.35	67.26	71.02	70.35
	5		67.23	68.58	68.74	68.10
	6		67.97	68.00	68.09	68.43
	7		68.06	68.18	68.60	67.57
Heading	4	7.VI	72.98	73.05	77.36	75.58
	5		71.61	72.31	73.10	71.99
	6		66.58	66.94	68.19	66.79
	7		64.02	64.16	64.58	64.25
	8		60.94	60.82	61.99	64.15
Flowering	5	17.VI	69.06	68.57	73.09	69.90
	6		72.17	72.88	73.45	75.45
	7		69.12	65.59	70.47	70.42
	8		63.17	63.65	68.95	69.02
Waxy stage	7	1.VII	31.87	34.98	49.54	57.00
	8		55.81	56.00	57.01	62.16

The data in Table 1 show that in the leaves of wheat plants subjected to a presowing treatment with succinic and fumaric acids there is more water than in leaves of control plants. Two maxima in water content were noted: the first occurred in the third leaf stage and the second in the heading stage. As a rule leaves of the upper nodes contain less water than leaves of the lower nodes. In the waxy stage, however, when leaves of the lower nodes had almost perished, the maximum water content occurred in leaves of the uppermost node.

Chlorophyll Dynamics

It has been established by various investigators that the quantity of chlorophyll in the leaves of annuals increases and reaches a maximum at the time of flowering [16-19]. The amount of chlorophyll in leaves of the various nodes of a single plant does not remain constant. For example, in spring wheat the chlorophyll concentration steadily increases in rhythm with the plant's development, and reaches a maximum at the last leaf budding stage of the head, while in unvernalized winter wheat, which remains in the tillering stage all summer, the chlorophyll content, having reached a maximum, remains constant till fall [20].

Mineral nutrients exert a significant influence on the accumulation of chlorophyll by plants [21]. It has been noted that different elements have different effects: potassium deficiency in flax induces a sharp reduction in chlorophyll content while a decrease in phosphorus level is associated with an increased chlorophyll content; in sugar, beet leaves increased doses of potassium and sodium salts lead to a reduction of chlorophyll content and increased doses of magnesium chloride and ammonium salts lead to an increase. Copper enhances the accumulation of chlorophyll in leaves and inhibits its destruction. With the application of organic-mineral fertilizers to rotation farms planted in grass, there was an increased chlorphyll content of leaves of winter wheat, sugar beet, kok-saghz and perennial grass mixtures [22].

It is known that with a temporary chilling of seedlings of mung bean (Phaseolus aureus Roxb.) their enzyme activity increases sharply [3], and in wheat seedlings there is a more rapid accumulation of chlorophyll [20]. These data indicate a possible connection between the formation of biogenic stimulators in chilled tissues and the subsequent increase in catalase activity and chlorophyll content. In view of the fact that succinic and other acids are components of the complex of biogenic stimulators, we devised an experiment to clarify the effect of temporary chilling of seedlings and soaking of seeds of Lyutestsens 62 wheat in succinic acid solutions.

Experiments were carried out as follows: 1) seeds were soaked in 10 ml of distilled water in Petri dishes and germinated at 20°; 2) seeds were soaked as before and after saturation at 20° were chilled for 4 days

TABLE 2

The Dynamics of Chlorophyll Accumulation in the Leaves of Lyutestsens 62 Wheat in Relation to Presowing Application of Stimulators (in % of absolute dry wt; nodes counted upwards)

Stage of development	Leaf nodes	Date of determination	Control—dry seeds	Control—seeds soaked in water	Seeds treated with succinic acid	Seeds treated with fumaric acid
Tillering	1—3 from the rootstock	5.V	2.06	2.08	2.61	2.63
Shooting	1	26.V	1.63	1.76	1.87	1.92
	2		1.61	1.65	1.87	2.10
	3		1.79	1.89	1.96	1.99
	4		1.70	1.61	2.04	1.77
Heading	1	7.VI	1.28	1.33	1.43	1.59
	2		1.75	1.85	2.40	2.49
	3		3.48	3.51	3.85	4.19
	4		2.87	2.85	3.17	3.03
	5		2.25	2.30	2.43	2.37
Flowering	2	17.VI	1.27	1.33	1.39	1.21
	3		1.70	1.84	2.51	2.47
	4		2.44	2.54	2.93	3.31
	5		2.49	2.55	3.80	4.17
Waxy seed stage	4	1.VII	0.20	0.19	0.37	0.30
	5		0.26	0.26	0.56	0.48

Note: In the tillering stage, leaves from the rootstock were taken for analysis and in all subsequent stages stem leaves were used.

TABLE 3

Crude Chlorophyll Content of Blades of Lyutestsens 62 Wheat Leaves at Various Nodes in Relation to Presowing Application of Stimulators (in % of absolute dry wt; notes counted upward)

Stage of development	Leaf nodes	Date of determination	Control—dry seeds	Control—imbibed seeds	Succinic acid, M/500
First leaf		21.IV	1.27	1.11	1.38
Third leaf	1	4.V	1.27	1.68	1.96
	2		1.31	1.22	1.48
	3		1.23	1.19	1.40
Tillering	1—3	15.V	1.28	0.96	1.25
	4		1.63	1.69	1.73
	5		1.77	1.85	2.86
Shooting	3	5.VI	0.82	1.28	1.33
	4		1.42	1.32	1.85
	5		1.68	2.03	2.47
	6		1.56	1.87	2.08
	7		1.36	1.55	1.90

at 0 to −3°; 3) seeds were exposed to 0.0002M succinic acid for a day and subsequently germinated under the same conditions as in treatment one; 4) after a twenty-four-hour exposure to succinic acid, and their saturation in warmth, seedlings were chilled as in treatment two. It is understood that in those seedlings which had not undergone chilling the roots and aerial organs of the plant grew markedly, while in chilled seedlings the length of these organs was perceptibly smaller. All seedlings were therefore kept in a thermostat at 20° for two days following chilling. Subsequently they were placed in the light. Seven days from the time of transfer of the seedlings to the light a determination of the crude unsaponified chlorophyll in the leaves was made by Gëtry's colorimetric method.

TABLE 4

Chlorophyll Content of Leaves of Odessa 10 Corn at Flowering of the Female Inflorescence in Relation to Presowing Treatment of the Seeds with 0.0002M Succinic and Fumaric Acids (in % of absolute dry wt; leaf nodes counted upwards)

Treatment	Leaf nodes							
	1	3	5	7	8	9	10	11
Control – dry seeds	0.53	–	1.17	1.60	1.59	1.58	1.71	1.34
Control – seeds soaked in water	0.91	1.20	1.41	1.62	1.55	1.55	1.53	1.12
Seeds treated with succinic acid	1.35	1.43	1.74	1.88	2.38	2.17	2.25	1.90
Seeds treated with fumaric acid	1.37	1.83	1.87	1.74	2.11	2.04	1.83	1.79

The experimental data show that with chilling of seedlings as with exposure of seeds to succinic acid a large quantity of chlorophyll accumulates in wheat leaves. The highest chlorophyll levels were observed where the seeds had been exposed to succinic acid and subsequently chilled. From this it may be concluded that as a result of chilling biogenic stimulators which promote chlorophyll accumulation are formed in the seedlings.

In the 1955 experiments, chlorophyll and associated pigments in the leaves of Lyutestsens 62 wheat, the seeds of which had been soaked for a day prior to sowing in 0.0002M dicarboxylic acids, were determined at the following stages – tillering, shooting, heading, flowering and waxy seed stage. Leaf samples were taken at 7-8 o'clock in the morning. In the tillering stage they were taken from the three upper leaves, and at succeeding stages they were taken from the stem leaves strictly according to nodal position, the nodes being counted upwards. The lowermost leaves, from the shooting stage onward, had in the majority of cases turned yellow and partially withered and were therefore not used for analyses.

Determination of crude chlorophyll was made, as in other experiments, by Gëtry's colorimetric method (Table 2).

In the 1956 experiments,the chlorophyll content of leaves at various nodes was investigated from the first leaf stage to the end of the shooting stage (Table 3). In the shooting stage the first and second leaves died.

The data summarized in Tables 2 and 3 show that a presowing treatment of wheat seeds with weak solutions of dicarboxylic acids exerts a stimulatory effect on chlorophyll accumulation. Externally these plants are a darker green than the controls. In the course of plant development two maxima in chlorophyll accumulation were noted: one occurred during the tillering stage and the other at the end of the heading and the beginning of the flowering stage, the maximum quantity of chlorophyll being found in the flowering period. Old leaves (at the lower nodes) and young leaves (at the upper nodes) contain less chlorophyll than leaves of the middle nodes, although there was an increase in chlorophyll content from one leaf to the next in an ascending direction throughout the growing period.

The determination of chlorophyll content in leaves of Odessa 10 corn at various nodes, where the seeds had received a presowing treatment with succinic acid solution, was carried out in the fourth leaf stage, on May 17, 1955, and in the flowering stage, June 30, 1955.

The content of "crude" chlorophyll (in % of absolute dry wt) at the fourth leaf stage of leaves of the first node (counting from the bottom) was 1.07%, of the second node – 1.07%, of the third node –1.08% and of the fourth node – 1.10% in plants grown from seeds sown dry; in plants grown from seeds soaked in distilled water, the amounts were, respectively: 1.26; 1.28, 1.28 and 1.23%; in plants grown from seeds soaked in 0.0005 succinic acid they were: 1.28, 1.35, 1.38 and 1.51%.

Data on "crude" chlorophyll content in the flowering stage are presented in Table 4.

TABLE 5

The Effect of Dicarboxylic Acids at Various Concentrations on the Activity of Catalase($k_{15°} \cdot 10^4$) Seedlings of Lyutestsens 62 Wheat

Molar concentrations of acids	Seedling age in days			
	1	3	8	15
Succinic acid				
Control – water	6.3	33.1	121.4	94.7
0.001 M	5.6	33.2	142.9	91.7
0.0002 M	7.5	44.6	141.6	94.8
0.0001 M	6.2	42.0	123.9	109.5
Fumaric acid				
Control – water	4.7	41.2	87.1	128.7
0.001 M	6.3	32.2	116.5	118.4
0.0002 M	8.0	44.7	121.2	143.9
0.0001 M	6.0	31.8	128.3	130.4

The data of Table 4 show that, as in wheat, the amount of chlorophyll in corn leaves, where there has been a presowing treatment with solutions of dicarboxylic acids, is greater than in controls. The maximal amount of chlorophyll is found in leaves of the middle nodes, i.e. in leaves nearer to the female inflorescence, while leaves of the upper and lower nodes contain less chlorophyll.

Change in Catalase Activity

Many investigators have studied catalase activity during plant ontogenesis. Special attention has been devoted to the change in its activity during seed germination [23-25]. The studies of Blagoveshchenskii and his students [3] have shown that young plants have more efficient enzymes than older plants. It was also shown that the activity and the quality of the enzymes increases in seedlings which have been subjected to a temporary chilling [26].

There is a body of data which indicates that indicates that the activity and efficiency of catalase and other enzymes is increased as a result of a presowing treatment of seeds with biogenic stimulators [3, 5, 7]. Thus, for example, the efficiency of catalase in sprouting mung bean was observed to be enhanced by very dilute solutions of succinic, fumaric and trans-cinnamic acids; l-malic and tartaric acids were found to activate trypsin at 0.0003M; in seedlings of sweet white corn, Melanopus 69 wheat and Pallidum 32 barley, the seeds of which had been soaked for twenty-four hours in very dilute solutions of succinic acid, an increase in catalase activity was observed together with a stimulation of growth processes [13].

We will present her the results of our investigations of catalase activity at 15 deg of dormant seeds and one-, three-, eight- and fifteen-day-old seedlings of Lyutestsens 62 wheat, the seeds of which were subjected to a 24-hour treatment with solutions of succinic and fumaric acids, and also results of investigations of activity of the leaves of the same wheat stimulated,prior to sowing,by 0,0002M solutions of dicarboxylic acids,in relation to developmental stage.

Catalase determination was carried out by a gasometric method [4]. For determination of catalase activity in dormant seeds and their seedlings, aliquots of 58 seeds weighing 2 g each were taken. Aliquots of leaf tissue from wheat plants in the field also weighed 2 g.

Seeds, seedlings and leaves, after maceration in 10 ml of phosphate buffer (pH, 6.98), were transferred to 100 ml volumetric flasks and made up to volume with distilled water. For each replicate 10 ml of this extract were placed in one sidearm of a catalase vessel and 3 ml of a 5% solution of H_2O_2 were placed in the other sidearm. Measurement of oxygen evolved were made every 30 seconds, or alternatively every minute. Experiments were run in triplicate.

Catalase activity (K) was calculated according to the equation for a monomolecular reaction:

$$K = \frac{2.3}{t} \lg \frac{a}{a-x},$$

where a is the quantity of oxygen evolved in the complete degradation of the peroxide present: x is the quantity of oxygen in cm^3 given off in a time (t) expressed in seconds.

The value of K in dormant seeds of Lyutestsens 62 is extremely low: at 15° $K = 4.4 \cdot 10^{-4}$. When germination has begun, however, the activity of catalase rises markedly. This is especially striking in the first 8 days of germination (Table 5).

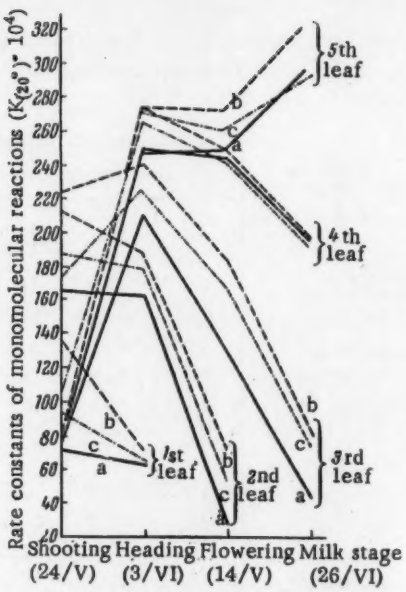
TABLE 6

Activity of Catalase ($k_{20°} \cdot 10^4$) in Leaves of Lyutestsens 62 Wheat in the First Leaf Stage and the Tillering Stage

Stimulators	First leaf stage	Tillering stage
Control (water)	37.7	125.2
Succinic acid	29.7	143.2
Fumaric acid	43.1	169.5

As is evident in Table 5, seedlings stimulated by succinic and fumaric acids have, as a rule, a higher catalase activity than control seedlings. In this connection it should be noted that the best results were obtained with 0.0002M solutions of succinic and fumaric acids.

With succinic acid the highest activity was found in 8-day-old seedlings, and with fumaric acid, with 15-day-old seedlings. This is due to the fact that in the first case the seedlings were grown throughout the experiment on distilled water.



Catalase activity of leaves of various nodes in Lyutestsens 62 wheat.
a — Control; b — succinic; c — fumaric acid

Under the influence of the stimulators, the respiration and rate of utilization of reserve materials by the seedlings was increased. At the end of the experiment these seedlings suffered from a mineral deficiency, and the change in catalase activity could only be attributed to this. The seedlings in the experiment with fumaric acid were grown on Knop's solution from the 8th day, however, and therefore catalase activity increased up till the end of the experiment.

In Table 6 and the Figure, data on catalase activity in the leaves of Lyutestsens 62 wheat at 20° expressed as the rate constant of a monomolecular reaction, are given in relation to presowing treatment with 0.0002M solutions of succinic and fumaric acids and in relation to developmental stage. It should be noted that determination of catalase in the tillering stage was made on leaves, from the rootstock and not according to nodal position as in subsequent stages.

From Table 6 it is clear that catalase activity is not affected in the same way by the two acids. In the first leaf stage it is reduced with succinic acid treatment and somewhat increased with fumaric acid treatment. In the tillering stage it increases markedly. In this period the experimental plants have a higher catalase activity than do the control plants. Here, as in the first leaf stage, the stimulatory effect of fumaric acid is greater than that of succinic acid. The stimulated plants are dark green in color, in contrast with the controls, and have undergone greater growth.

As the figure shows, a presowing treatment with succinic and fumaric acids enhances the catalase activity of the stem leaves.

From an analysis of the data presented it is obvious that catalase activity in leaves of spring wheat continues to increase in an ascending direction throughout the plant's development and reaches a maximum in the uppermost leaf at the milk stage. Moreover, it was observed that the catalase activity of a given leaf increases as the leaf grows and is maintained at a high level until the cessation of growth of the next higher leaf. It is probable that the high activity of catalase of leaves of individual nodes reflects their physiological significance at a given period in the plant's development.

SUMMARY

Treatment of seeds of spring wheat with solutions of succinic and fumaric acid in optimal concentrations exerts an effect on catalase activity, chlorophyll content and water content of leaves. Catalase activity in ex-

perimental wheat plants remains higher throughout the growth period than in control plants under the same conditions, as a rule. From the moment of germination the catalase activity increases uninterruptedly in an ascending direction from the leaf of a given node to the leaf of the next higher node, and reaches a maximum in the milk stage in the two upper stem leaves.

Spring wheat and corn plants pretreated with succinic and fumaric acids contain more unsaponified chlorophyll than control plants. During the growing period the maximum content of chlorophyll in the leaves occurs at tillering and in the heading-flowering stage. Water content of leaves of stimulated plants is as a rule higher than that of the controls.

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CHEMICAL DEFOLIATION OF FRUIT TREES

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It is known that autumnal leaf fall of fruit trees is often far from being accomplished before the onset of frost. Frost-killed leaves in which, up to this time, an abscission layer has still not yet formed, lose moisture quickly and remain on the plant in the dry condition for a long time. Dry leaves left on the branches facilitate the distribution of pests and disease and severely reduce the effectiveness of insectofungicides applies to the trees. Moreover, the presence of large numbers of dry leaves in the crown facilitates the accumulation of snow in the crown and this creates the hazard of limb breakage. There are also observations which indicate that suppression of autumnal leaf fall delays the preparation of the leaves for over-wintering and gives them less resistance to low temperature [1, 3].

The foregoing indicates the necessity to work out practices which would assure the fall of leaves of fruit trees all at the same time and still would not exert some kind of negative effect on them.

It is also necessary to employ such practices in fruit nurseries before the autumnal lifting of stock since wholesale lifting of stock, as a rule, occurs just before they drop their leaves. Hand stripping the leaves abruptly decreases water loss by the stock and also increases their resistance during transport and storage in holding beds [2]. However, hand defoliation of stock is not only ideal but is also a very difficult job to do.

Naturally, the question arises whether it would be possible to use as agents for defoliation of fruit trees chemical preparations already being widely used for the preharvest defoliation of cotton and a number of other plants [4, 7]. It should be noted in this connection that a few orientative experiments, which have yielded positive results [8-13], have already been conducted on defoliation of fruit trees and ornamental plants.

We conducted a large part of these experiments in the nursery and on the plots of fruit-bearing trees of the Sovkhoz Meshkovo near Moscow. In addition, in a number of experiments two and three year old sets were used, which were raised in greenhouse containers under greenhouse and conservatory conditions in the K. A. Timiryazev Institute of Plant Physiology, Academy of Sciences USSR.

The experimental plants were: apple sets — Grushovka moskovskaya, Pepin shafrannyi, Antonovka obyknovennaya, Slavyanka, Severnyi sinap and Yubileinoe; Pear — Bessemyanka, Tonkovetka, and Malogopzhatka; cherry — Vladimirovskaya, Lyubskaya, and Shirpotreb; fruit-bearing apple trees — Grushova moskovskaya, Pepin shafrannyi, Antonovka obyknovennaya, and Shtreifling; pear — Bessemyanka and Tonkovetka; and cherry — Vladimirovskaya and Lyubskaya.

As defoiliants we used aqueous solutions of magnesium chlorate (hexahydrate) and sodium pentaborate (0.125; 0.25, 0.5; 1 and 2%) and Endothal (disodium salt of 3, 6-endoxohexohydrophthalic acid) in concentrations of 0.05; 0.075, 0.1; 0.25; 0.5; 1 and 2%).

The work was carried out from 1956-1958 inclusive. Different plant defoliation times were compared in setting up the experiment (August, September, and October). Ten to fifty plants (sets) were used in each experimental treatment under nursery conditions. One-year old sets were sprayed with 1000 liters and the two-

TABLE 1

Leaf Fall from Fruit Sets and Trees Treated with Magnesium Chlorate and Endothal (treated August 28, 1957)

Crop and defoliant	Sol. conc. %	Leaf fall on 12th day, %	Crop and defoliant	Sol. conc. %	Leaf fall on 12th day
Apple sets			Fruit-bearing apples		
Grushovka Moskovskaya			Grushovka Moskovskaya		
Magnesium chlorate	0.25	94.5	Magnesium chlorate	0.25	96.9
Endothal	0.075	89.1	Endothal	0.075	85.0
Pepin shafrannyi			Pepin shafrannyi		
Magnesium chlorate	0.25	98.0	Magnesium chlorate	0.25	90.5
Endothal	0.075	94.8	Endothal	0.075	85.0
Anatovka-obyknovennaya			Anatovka-obyknovennaya		
Magnesium chlorate	0.25	85.0	Magnesium chlorate	0.25	87.0
Endothal	0.075	89.0	Endothal	0.075	90.3
Shtreifling			Shtreifling		
Magnesium chlorate	0.5	93.5	Magnesium chlorate	0.5	79.1
Endothal	0.1	90.8	Endothal	0.1	85.0
Pear sets			Fruit-bearing pears		
Bessemyanka			Bessemyanka		
Magnesium chlorate	0.25	50.1	Magnesium chlorate	0.25	49.0
Endothal	0.075	93.2	Endothal	0.075	97.8
Tonkovetka			Tonkovetka		
Magnesium chlorate	0.25	49.0	Magnesium chlorate	0.25	70.5
Endothal	0.075	96.3	Endothal	0.075	95.0
Cherry sets			Fruit-bearing cherries		
Vladimirskaya			Vladimirskaya		
Magnesium chlorate	0.25	40.1	Magnesium chlorate	0.25	30.9
Endothal	0.075	90.7	Endothal	0.075	87.1
Lyubskaya			Lyubskaya		
Magnesium chlorate	0.25	60.9	Magnesium chlorate	0.25	40.7
Endothal	0.075	98.0	Endothal	0.075	99.1

* The leaf fall of control plants was not observed at the time that treatment results were accessed.

year old sets with 2000 liters of the defoliants per hectare of planted area. Two or three trees (planted 1939 and 1949) were taken for each treatment in the experiments with fruit-bearing trees. Half of the crown of each tree was treated and the other half served as a control. The normal outlay of working solution was five liters per tree of the 1949 planting and 10 liters per tree of the 1939 planting.

The data on leaf fall from sets and fruiting trees with optimal treatment times and defoliant concentrations (magnesium chlorate 0.25; 0.5 and Endothal 0.075 and 0.1%) are presented in Table 1.

It is evident from Table 1 that a 0.25% solution of magnesium chlorate is a highly effective defoliant for all varieties of apples and their sets (except winter variety Shtreifling). The most effective defoliant for pears and cherries seems to be a 0.075% solution of Endothal.

It should be noted that the leaves of treated plants fell before the onset of frosts while leaf fall of the control plants principally occured after the frosts. The leaves of certain early varieties fell with a lower concentration of defoliant solutions than did those of later maturing varieties.

TABLE 2

Effect of Magnesium Chlorate and Endothal on Chlorophyll Content of the Leaves of Pepin shafrannyi Apple Sets (mg/ g dry wt.) (treated 10 A. M. August 18, 1957, analytical samples taken at noon)

Expt. treatment	18.VIII			19.VIII			20.VIII			21 VIII			22.VIII			23.VIII (fallen)		
	total	a	b	total	a	b	total	a	b	total	a	b	total	a	b	total	a	b
Control	6.24	4.46	1.77	7.55	5.58	1.97	6.26	4.66	1.60	6.45	4.88	1.57	6.75	5.10	1.65	7.77	5.49	2.28
Magnesium chlorate 0.25%	5.17	4.54	1.63	4.98	3.85	1.13	4.30	3.30	1.00	4.13	2.68	1.45	4.00	2.68	1.32	4.03	2.78	1.25
Endothal 0.075%	4.93	3.74	1.19	4.57	3.23	1.34	4.30	2.82	1.48	3.86	2.29	1.57	4.97	3.60	1.37	4.71	3.13	1.58

TABLE 3

Effect of Magnesium Chlorate and Endothal on the Amount of Forms of Water in the Leaves of Pepin shafrannyi Apple Sets % of Leaf Dry weight)

Expt. treatment	3th day			4th day			5th day			6th day (fallen)		
	total	free	bound	total	free	bound	total	free	bound	total	free	bound
Control	61.0	49.6	11.4	57.82	55	2.8	58.81	54.9	3.9	59.44	46.2	13.2
Magnesium chlorate 0.25%	58.86	40.5	18.3	41.43	16.9	24.5	28.32	0	18.32	30.60	0	30.6
Endothal 0.075%	56.86	41.1	15.7	57.75	44.9	12.8	53.86	31.7	22.1	50.58	30.58	20.0

In Table 2 we are presenting the results of analysis which concern the effect of magnesium chlorate and endothal on the chlorophyll content of apple leaves. The chlorophyll was extracted with ethyl alcohol and then determined with a spectrophotometer.

The data of table 2 shows that magnesium chlorate and endothal decrease the amount of chlorophyll a and b, the amount of the former being appreciably greater than the amount of the latter. An abrupt decrease of chlorophyll b in the leaves was already observed two hours after plant treatment with the defoliants. The minimum chlorophyll content of the leaves occurred at the time of leaf fall.

During the first day after plant treatment with a defoliant the water retention ability of the leaves decreases, the transpiration increases and, beginning with the second day, transpiration decreases while the water retention ability of the leaves increases.

The change in the forms of water in the leaves under the influence of defoliants is presented in Table 3 (the determination was made by the refractrometric method of Marinchuk). It is apparent from Table 3 that the more the total water content of the leaves decreases, the more the bound water content increases. On the fifth day all of the water is found in the bound state in the leaves of plants treated with magnesium chlorate. In plants treated with magnesium chlorate the decrease in transpiration and increase in water retention ability of the leaves set in more quickly than in plants treated with endothal. Consequently, these processes are closely linked with a decrease of free water content and an increase of bound water in the leaves.

We also set up an experiment concerned with the effect of defoliants on the water content of various plant organs.

Water content was calculated after leaf fall. Plants raised in greenhouse vessels were treated with magnesium chlorate on August 28 and not irrigated for 10 days after leaf fall. Analyses were made at the end of this period. Leaf fall began on the sixth, and was complete on the twelfth, day after spraying.

TABLE 4

The Effect of Defoliation on Water Content (%) of Different Plant Organs of Pepin shafrannyi Apple Sets.

Expt. treatment	Shoots	Stems	Roots, 1st, 2nd, 3rd order	Rootlets and root hairs
Control (with leaves)	39.4	34.6	33.0	35.0
Magnesium chlorate treatment 0.25%	47.4	41.5	43.5	47.9

Table 4 shows that plants subjected to defoliation were more rich in water than the control plants. It appears from this that leaf fall facilitates water conservation in the plant tissues. It was also shown that on the 20th day the amount of water in untreated plants with leaves and in plants defoliated by hand (on which about 40% of the leaves were left) was 7-8% less than plants treated with the defoliants (without leaves).

Experiments were made on two-year old sets of apple variety Pepin shafrannyi in order to assess the effect of defoliants in the water content of the soil layer occupied by roots.

Plants were raised in greenhouse vessels containing eight kilograms of soil. Vessels in which the soils had the same moisture content were selected for the experiments. Experiments were set up at the end of September in the greenhouse. The crowns of the sets in the experimental treatments were sprayed with a 0.25% solution of magnesium chlorate. Leaf fall from treated plants began on the sixth and was finished on the twelfth day after spraying. Soil moisture was determined on the twentieth day after treatment with a defoliant solution. Soil samples were taken to a depth of 15 cm.

The analyses showed that the soil moisture of the experimental treatment was 15-18% higher than the moisture of the control (control 10%, experimental treatment 37-38%). These data indicate that in causing leaf fall and curtailing transpiration, the treatment of plants with defoliants facilitates conservation of soil moisture.

Part of the potted plants indicated above were left to overwinter in an unheated open box. A determination of plant condition made after they overwintered showed that all control plants were very severely damaged and died while the plants treated with defoliants remained alive and then developed normally. Consequently, with higher soil moisture the plants were more winter resistant. It was shown, with the aid of a fluoroglucein solution, that defoliation facilitates shoot lignification in that lignification is somewhat more rapid in winter and fall than in summer varieties.

The effect of chemical defoliation upon frost resistance of mature trees and sets of different varieties was also explained in our investigations.

One-year old apple shoots 30-35 cm long were cut for the determination of frost resistance. Experiments in this direction began in 1957 and 1958 during the period from January to April. Then they were hardened. The latter consisted of holding the shoots for two days at −5° and then for three days at −10°. The hardened shoots (in groups of 10) were placed in cabinets at temperatures of −30, −35, −40, and −50° for one day. The hardening and chilling of the shoots was done in the special refrigeration chambers of the artificial climate station of the Timiryazev Institute of Plant Physiology, Academy of Sciences, USSR. After chilling the shoots were left at +2° for one day. Then they were placed in vessels with water and transfered to the hothouse for further observations. The degree of shoot frost resistance was judged by the number of buds which opened.

The observations made show that plants treated with defoliants at an optimal time (first half of September) do not differ from control plants in their frost resistance, and the frost resistance of certain autumn and winter varieties (Pepin shafrannyi and Shtreifling) is somewhat greater in the case of treatment with defoliants.

SUMMARY

Aqueous solutions of magnesium chlorate and endothal can be employed for fall defoliation of fruit trees (saplings and fruit bearing trees). In the Moscow region the most profitable time for defoliation is the first half of September. At this time a 0.25%–0.5% solution of magnesium chlorate and a 0.075–0.1% solution of endothal should be used.

Chemical defoliation is a highly effective method and can be employed instead of stripping of the leaves of fruit saplings in arboretums. The new method can also be used for fall defoliation of fruit bearing trees. It improves the readiness of the plants for wintering and increases the efficiency of fall and winter measures against sickness of the plant and against garden pests.

Spraying of trees with solutions of defoliants not only accelerates the formation of abscission layers in petioles but also accelerates lignification of the shoots, lowers transpiration, facilitates retainment of water in the plant and in the root zone in the soil, and makes the plants more resistant to low temperatures during the winter period.

Observations made after wintering of the plants showed that, with respect to condition, growth and development (leaf area, and chlorophyll content, rate of branch growth, nature of flowering, setting and formation of fruits, quality and size of crop), plants subjected to defoliation practically do not differ from the control plants.

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THE POSSIBILITY OF TRANSFORMATION OF CARBANILIC ESTERS IN PLANTS

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Investigations conducted recently in the field of insecticides acting within the plant have tended to indicate that certain of these insecticides, in particular Shradan, isosystox, parathion, etc., after entering the plant are transformed into more active products of the oxidative metabolism, which are quite soluble in water and are readily translocated in the plant [1, 2, 3]. A similar kind of observation has also been made with the herbicide group of arylhydroxyalkylcarbonic acids [4] and in other cases [5].

A number of facts described in the literature indicate the possibility of a metabolic conversion of the esters of phenylcarbamic acids in plants. For example, a careful study of herbicide residues in a diversity of agricultural crops treated with isopropylphenylcarbamate (IPC) and isopropyl-N-3-chlorphenylcarbamate, which was carried out using a highly sensitive colorometric method, indicated the complete absence of even traces of the unaltered material in the plants [6, 7]. Moreover, a special investigation has shown [8] that when IPC is applied in the form of a lanolinic paste on the surface parts of the plants, the preparation does not enter and is not transformed in the plants.

With the aim of tracing possible first products of the transformation in plants of IPC, which has a high activity and easily penetrates and is translocated in plants, we first studied the physiological activity of all possible isomeric oxididation products of IPC. Until recently the herbidical activity of such types of compounds had still not been studied.

The results of initial tests on wheat seedlings, the results of which are presented in Table 1, showed that the introduction of a hydroxyl group into the IPC aromatic nucleus exerts such an influence in the herbicide activity of the compound that second order substitutions [9]: ortho- and metahydroxy products of IPC exerted approximately one-thousandth the activity of the original compound and the parahydroxy products was practically inactive.

In contrast to this N-hydroxyisopropylphenylcarbamate (N-hydroxy IPC) was a highly active herbicide with an activity close to IPC.

Initial tests with other simple esters of N-hydroxyphenylcarbamic acid on wheat seedlings showed that in this series, analogously with the esters of phenylcarbamic acid, the isopropyl ester had the greatest activy and all other esters studied showed little activity. The low activity of N-metahydroxyisopropylphenylcarbamate shown in the experiments with wheat seedlings also bears witness to a connection between the high physiological activity of N-hydroxy IPC and the presence of a free hydroxyl group in this compound with nitrogen.

In order to study more deeply the herbicidal activity of N-hydroxy-IPC and also to investigate the nature of the selective effect and the ability of N-hydroxy-IPC to penetrate the leaves and exert a phytotoxic effect, we conducted experiments with N-hydroxy-IPC on vegetative oats and radish plants. The seedlings and the soil along with them were sprayed with 0.040-0.4% solutions of the preparation. For comparison a parallel analogous treatment of plants was made with a 0.04% solution of IPC.

TABLE 1

Growth Retardation of Wheat Seedlings with Hydroxy Products of Esters of Phenylcarbamic Acid

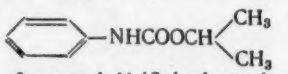
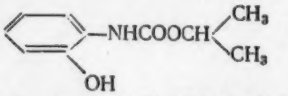
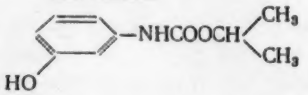
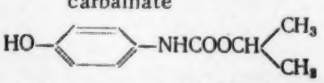
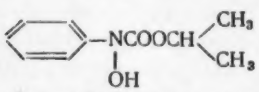
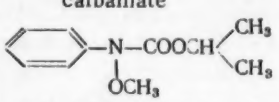
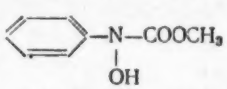
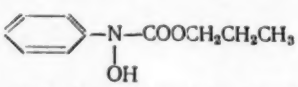
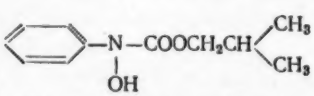
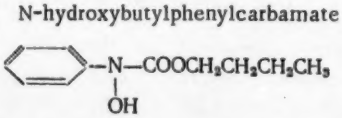
Prep. No.	Compound and structural formula	Methyl point in °C or boiling point in °C/ mm	Conc., %	Average length, % of control	
				culm	roots
1.	Isopropylphenylcarbamate 	90	10^{-4}	20.5	12.1
			10^{-3}	22.5	11.5
			10^{-2}	Seeds did not germinate	
2.	Isopropyl-N-(2-hydroxyphenyl)-carbamate 	87.5	10^{-4}	109	95
			10^{-3}	114	95.8
			10^{-2}	104	90.0
			10^{-1}	Seeds swelled, no roots	
3.	Isopropyl-N-(3-hydroxyphenyl)-carbamate 	80.5	10^{-4}	109	136.4
			10^{-3}	116	97.8
			10^{-2}	106	95.0
			10^{-1}	Seeds swelled, no roots	
4.	Isopropyl-N-(4-hydroxyphenyl)-carbamate 	112	10^{-4}	118	136.9
			10^{-3}	125	153.1
			10^{-2}	106	106.6
			10^{-1}	60.5	60.5
5.	N-hydroxyisopropylphenylcarbamate 	58	10^{-4}	103	92.4
			10^{-3}	37	43.5
			10^{-2}	23	16
			10^{-1}	20.7	14.3
6.	N-metahydroxyisopropylphenyl-carbamate 	82—83/0.15	10^{-4}	89.0	100.3
			10^{-3}	94.3	100.5
			10^{-2}	87.7	90.4
			$2{,}5 \cdot 10^{-2}$	48.3	41.3
7.	N-hydroxymethylphenylcarbamate 	120—122/0.5	10^{-4}	108.3	112.0
			10^{-3}	103.0	106.3
			10^{-2}	97.2	100.0
			10^{-1}	72.5	47.3
8.	N-hydroxypropylphenylcarbamate 	134—136/0.6	10^{-4}	107.5	119.0
			10^{-3}	109.5	119.1
			10^{-2}	100.0	106.7
			10^{-1}	33.1	21.2
9.	N-hydroxyisobutylphenylcarbamate 	22—24 132—135/0.5	10^{-4}	102.2	109.1
			10^{-3}	111.3	120.8
			10^{-2}	102.3	112.5
			10^{-1}	Seeds did not germinate	
10.	N-hydroxybutylphenylcarbamate 	142—143/0.4	10^{-4}	96.1	103.0
			10^{-3}	93.0	91.2
			10^{-2}	84.0	67.2
			$5 \cdot 10^{-2}$	40.0	37.0

Fig. 1. Oat plants 10 days after spraying with IPC and N-hydroxy-IPC solutions during the emergence stage (percent of dead plants shown in parentheses).
K – Untreated control: 1 – IPC, 0.44% (30%); 2 – N-hydroxy-IPC, 0.04% (35%); 3 – N-hydroxy-IPC, 0.1% (64.7%); 4 – N-hydroxy-IPC, 0.2% (76.4%); 5 – N-hydroxy – IPC, 0.4% (94.1%).

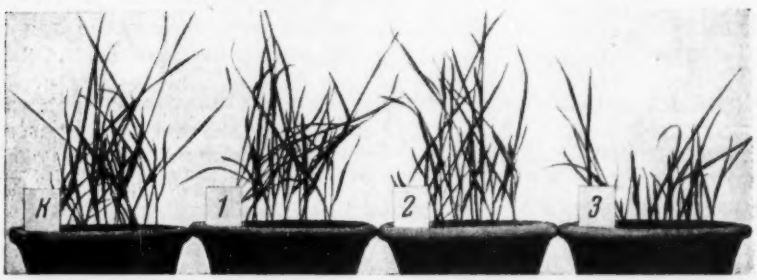


Fig. 2. Oat plants 10 days after spraying with IPC and N-hydroxy-IPC solutions during the emergence stage (soil covered with cotton bats during treatment).
1 – IPC, 0.04%; 2 – N-hydroxy-IPC, 0.04%; 3 – N-hydroxy-IPC, 0.2%.

Fig. 3. Radish plants 10 days after spraying with solutions of IPC and N-hydroxy-IPC during the emergence stage.
K – Untreated control: 1 – IPC, 0.04%- 2 – N-hydroxy-IPC, 0.04%; 3 – N-hydroxy-IPC, 0.1%; 4 – N-hydroxy-IPC, 0.2%; 5 – N-hydroxy-IPC, 0.4%.

Spraying oat plants with the preparations in the designated doses did not cause any kind of rapid morphological change. No burns, leaf yellowing, drooping, or contortion were observed, neither could the sprayed plants be distinguished from control plants on the second or third day after treatment. However, on the fourth or fifth day an abrupt retardation was observed in the growth of a number of the treated plants. The treated plants lost the ability to form leaf ranks and remained in the two-leaf stage (the second leaf was suppressed, its leaf blade was one-third to one-quarter as long as in the control). On the seventh and eighth day after treatment the leaf

TABLE 2

The Effect of Postemergence Treatment with IPC and N-hydroxy-IPC on Radish Growth

Preparation	Concentration %	Wet wt., % of control *	
		aerial parts	roots
IPC	0.04	108	102.5
N-hydroxy-IPC	0.04	111.5	110.5
	0.10	91.4	123.1
	0.20	107.7	116.4
	0.40	66.5	59.5

* Average weight of roots in control treatment approximated 28.2g/ pot (17 plants) and average weight of aerial parts, 44.0g.

tips yellowed and about fifteen to twenty days after treatment the leaves died completely. Figure 1 shows the conditions of the plants on the tenth day after treatment.

As shown by the experiments conducted, on the basis of their effect on plants (kind of dying and morphological changes), IPC and N-hydroxy-IPC are indistinguishable from one another. The somewhat greater herbicidal effect of N-hydroxy-IPC, in our opinion, is explained in that the compound is able to enter the plant,both through the root and the leaves.

A special postemergence experimental treatment of oat plants with IPC and N-hydroxy-IPC was carried out in order to verify this result. In order to exclude the preparations from the soil when the seedlings were sprinkled the soil was covered with cotton batting, which was then removed. In this experiment a definite herbicidal effect was observed only in the case where a 0.2% solution of N-hydroxy-IPC was used(Fig. 2).

That such a comparatively high concentration is needed for expression of the physiological activity of the preparation is explained by the small leaf surface of oat seedlings during the treatment period.

The N-hydroxy-IPC retains the selective herbicidal effect with respect to grasses characteristic for IPC.

As evident from Figure3 and the data from the experiment with radish presented in Table 2, only in the case where a 0.4% solution of N-hydroxy-IPC was used was there observed a noticable decrease in the weight of rhizomes and aerial parts of the radish.

It is interesting to note the appreciable (up to 23%) increase in rhizome yield after spraying oat seedlings with 0.1-0.2% solutions of N-hydroxy-IPC.

Experimental data obtained in trials on sprouting wheat and vegatative plants of oats and radish show that on the basis of strength, characteristics, and selectivity the herbicidal effect of N-hydroxy-IPC is very close to that of IPC, being distinguished from IPC by its greater solubility in water and also by its ability,upon foliar application,to penetrate and be translocated in the plant.

All of this to a certain degree supports the hypothesis advanced by one of the authors and Mel'nikov [9] that the esters of phenylcarbamic acid are rendered metabolically active by this oxidation to N-hydroxy- products. A hydroxyl group with nitrogen not only increases the solubility of the compound in water and permits it to penetrate the plant more rapidly but, apparently, is one of the reaction centers of the molecule, by which the herbicide reciprocally reacts with the vitally important plant substrates. In this connection it might be pointed out that in the absense of an herbicidally active ethyl-N, the formation of N-hydroxy-products of N-diphenylcarbamate [10] in plants is difficult.

A. Synthesis of Preparation

The derivation of hydroxy-products of the isopropyl ester of phenylcarbamic acid and alkyl esters of N-hydroxyphenylcarbamic acid has been described earlier [9].

Isopropyl ester of N-metahydroxyphenylcarbamic acid.

N-hydroxyisopropylphenylcarbamate, 16.2g, methyl alcohol, 50 ml, and methyl iodide, 18 g were mixed in a three-necked flask. To the mixture was gradually added,from a dropping funnel over a 5 hour period,a solution of sodium methylate previously obtained from 2.1 g of metallic sodium and 75 ml of methyl alcohol. After addition of the sodium methylate was completed,the mixture was warmed in a water bath for 2 hours, the

methyl alcohol was driven off, 200 ml of ether was added to the residue, the precipitated sodium iodide was filtered out, and the ether solution was extracted with a 5% aqueous solution of sodium hydroxide, washed with water and dried with magnesium sulfate. The dried ether solution was distilled and the residue driven over, under vacuum. The yield of N-metoxyisopropylphenylcarbamate was 15g, b. p. 82-83°/0.15 mm, $n^{20}D = 1.5082$.

0.1870 and 0.1908 g of the material was consumed in fixing 9.23 and 9.47 ml of 0.1N ammonium chloride. N found: 6.91 and 6.95%. $C_{11}H_{15}O_3$. Calculated N: 6.7%.

The analogously obtained isopropyl ester of N-ethoxyphenylcarbamic acid: yield 78g, b.p. 92/0.1 mm, $n^{20}D = 1.4998$. 0.1804 and 0.1889 g of the material was consumed in fixing 8.23 and 8.77 ml of 0.1N ammonium chloride. N found: 6.39 and 6.50%. $C_{28}H_{17}O_3$. Calculated N: 6.28%.

B. Biological Experiments

In the initial tests on wheat seedlings an equal number of seeds of wheat were soaked for 24 hours in solutions of the preparations at appropriate concentrations, washed off, and then germinated on filter paper in Petri dishes. After three days the length of the shoots and roots was measured. In Table 1 the data are presented from two parallel experiments expressed as percent of the control.

Oats variety Orel and radish variety Saksa were taken for study of the physiological activity of IPC and N-hydroxy-IPC on vegetative plants. The plants were grown in soil in tubs under greenhouse conditions. Soaked seeds were sown. Aqueous solutions of the preparations were sprayed onto the plants treated with a hand atomizer at a rate of 5 ml of solution per tub. Wetting agent OP-7 at a concentration of 0.1% was added to all solutions of the preparation. The plants were in the emergence stage (calendar age 4 days) at the time of treatment. The experiments were replicated 3 times.

The experiments with oats were terminated in 30 days, after which the number of dead plants was determined. In the experiments with radishes at the end of the vegetative stage, 30 days after treatment the plants were harvested and wet weight of the aerial parts and roots was determined. The data obtained from the radish experiment, the average of the three replicates expressed as percent of the control, is shown in Table 2.

SUMMARY

A high activity of hydroxyisopropylphenylcarbamate (N-hydroxy-IPC) was found during a study of the herbicidal action of hydroxy-derivatives of isopropylcarbamilic acid ester (IPC) on wheat seedlings. Other isomeric hydroxy-derivatives and the simplest hydroxycarbamilic acid alkyl esters were weakly active.

With respect to intensity, character and selectivity of its herbicidal action, N-hydroxy-IPC sprayed on oat and radish plants after sprouting was found to resemble IPC. However, in distinction to IPC, N-hydroxy-IPC deposited on oat leaves is capable of penetrating into the plant and exerting a herbicidal effect.

It is suggested that N-hydroxy-IPC is formed in plants as a product of biological activation of IPC.

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QUALITY OF THE SPRING WHEAT CROP AND THE POSSIBILITY OF FORECASTING IT FROM LEAF NITROGEN CONTENT

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A search for causes for the variability in the nitrogen content of wheat grains as an index of quality has guided the work of a number of investigators [1-3].

According to the data of Petrov [2] and Darkanbaev [3] the fundamental reason for the variation in amount of nitrogenous substances in wheat grains is the condition of the parent plant, which is determined by the tempo of its developmental stages and the effect of the external environmental factors.

The foliage [3-7] has the most important role in accumulation of nitrogenous materials in the wheat grain, the yield and quality of which depends upon the direction of biochemical processes in the leaves [8, 9]. Therefore, endeavors to establish a relation between nitrogen of the leaves or the entire plant and the grain nitrogen were made long ago. It has been established that during dry years plants contain relatively more nitrogen in the leaves, stems and also in the grain.

During moist years or under irrigation the nitrogen content of these organs is diminished. The reasons for this dependence has been disclosed in the works of Pryanishnikov [10], Petinov [5, 11, 12] and Petrov [2]. The work of investigators recently [13-16] shows the presence of an almost direct relation between protein nitrogen in the leaves and the grain. A relationship between leaf and grain nitrogen was not always observed in the work of other investigators [9, 17], therefore, the question of the connection between nitrogen content of the grain and the leaves is still not conclusively resolved. In our opinion, this is because the leaves of only a single storey were analyzed [5, 12, 15], which apparently, was insufficient for definite results or these same samples of all the leaves were picked during the period of their rapid growth and development, which might conceal a connection existing between leaf and grain nitrogen [14, 16].

The task of this work was to study the relation between the relative nitrogen content of wheat grain and vegetative leaves of the plants during the flowering to milk stage, and to assess the possibility of using this leaf analysis for controlling the nitrogen nutrition of spring wheat.

The flowering to milk stage was selected because toward this time the plant curtails leaf and culm growth, completes phasic development, completes phosphorous and potassium accumulation [18] and nearly completes nitrogen accumulation [2, 18, 19] in its organs.

The amount of the mobile form of soil nitrogen toward flowering time decreases very strongly [15], and for filling the grain the plant uses chiefly nitrogen accummulated earlier by reutilization of it from its vegetative organs which have completed growth [20]. Therefore, the total nitrogen content of the plant during this period [13], and especially of the vegetative leaves, indicates the plant nitrogen supply and indicates the possibility of forming grain of a certain quality [21].

Toward the time of the flowering-milk stage the lower leaves usually dry up, and the role of the middle and upper stories in the nutrition of the plant and filling of the grain is greatly increased [18, 22, 23]. The first four leaves, counting from the spike, have the greatest physiological activity during this period, and, since the yield

TABLE 1

Soil Moisture Trend (%) in Sowings of Wheat Variety Lyutestsens 62 (1954)

Horizon, cm	Before Sowing, May 10	Tillering, June 24	Flowering, July 6	Beginning milk stage, July 15	Beginning of wax stage, July 24
18–20	11.8	9.1	7.8	6.6	16.7
38–40	–	7.9	6.5	6.3	7.7

Note: Permanent wilting precentage of soil 5.9%.

TABLE 2

Total Nitrogen in Leaves and Grain of Wheat (% of oven dry wt.)

Treatment	Leaves				Grain	
	tillering	flowering	milk stage	fully mature	% nitrogen	% protein
	Lyutestsens 62					
	4.11	3.32	1.88	1.14	3.32	17.61
$N_{45}P_0K_{45}$	4.49	3.59	2.00	1.15	3.47	18.58
$N_{90}P_{45}K_{45}$	4.40	3.52	1.87	1.16	3.55	18.70
P_{54}	4.05	3.37	1.80	1.10	3.37	17.84
	Gordeiforme 10					
Unfertilized	4.10	3.38	1.97	1.33	3.38	18.00
$N_{45}P_{45}K_{45}$	4.60	3.68	1.89	1.39	3.42	18.24
$N_{90}P_{45}K_{45}$	4.40	3.74	1.76	1.48	3.54	18.92
$N_{45}P_{90}K_{45}$	4.46	3.71	1.82	1.39	3.49	18.70

of spring wheat under the conditions of western Siberia is determined by the development of the main culm, the leaves of the main shoots were analyzed.

METHODS

The work was conducted at the S. M. Kirov Agricultural Institute in Omsk during 1954-1957, in the field and greenhouse, using three varieties of spring wheat: Lyutestsens 62, Mil'turum 553 and Gordeiforme 10*

The climatic conditions of 1954-1957 were distinguished by the great dryness of the spring and the first half of the summer; 1956 was more moist. The experiments were conducted on an ordinary sandy chernozem of average development. They were replicated three times on 100 m^2 plots. Plant samples were picked at 11-12 A. M. during the flowering to milk stage (6-9 days after complete spike formation). There were 300-450 plants in each sample. The plants were held in distilled water for 20 minutes and then the leaf blades were separated from the stems and air-dried. The four top leaves were used for analysis (in Lyustestsens 62 and Mil'turum 553 the fourth, fifth, sixth, and seventh leaf beneath, in Gordeiforme 10 the sixth, seventh, eighth, and ninth leaf). The bottom first, second and sometimes also the third leaf at the time of sampling was usually dried up and functionless.

The greenhouse experiment was conducted in vessels holding 5.5 kg of oven dry soil. The experiment was replicated three times.

* The work was carried out in the Agronomy faculty under the direction of professor A. Z. Lambin.

TABLE 3

Total Nitrogen in Leaves and Grain of Mil'turum 553 Wheat

Treatment	1954		1955	
	Leaves	Grain	Leaves	Grain
Unfertilized	3.42	3.35	3.46	3.08
$N_{45}P_{45}K_{45}$	3.90	3.49	3.62	3.14
$N_{90}P_{45}K_{45}$	4.00	3.57	3.61	3.15

TABLE 4

Amount of Mobile Nutrient Substances in the Soil of Experimental Plot Prior to Sowing of Wheat (mg/kg soil dry wt) and Soil Moisture Change (%) in 0-40 cm Layer During Developmental Phases (1956)

Crop	Variety	$N\text{-}NO_3$	P_2O_5	K_2O	Emergence, May 18	Tillering, June 18	Flowering, July 13
1. Oats	Mil'turum 553	8.1	115.1	123.8	21.0	15.4	12.0
2. Maize	Lyutestsens 62	22.6	187.8	119.0	16.8	13.2	9.6

Note: P_2O_5 determined after Truog, K_2O after Brovkin.

Before the vessels were filled 50 mg of nitrogen and phosphoric acid and 100 mg of potassium oxide ($N_1P_1K_1$) per kilogram of soil was added. In individual treatments the amount of nitrogen and phosphoric acid was increased several times. To certain vessels 50-100 mg of nitrogen was applied at the time when the third and fifth leaves appeared. The experiment was conducted at 80% of field capacity in 1955, 60% in 1956, and 60 and 80% in 1957.

Fifteen plants were raised in each vessel. During the flowering to milk stage, three plants were cut from each vessel and the required leaves of the main shoot were anlyzed. Total and nonprotein nitrogen in the leaves and grain was determined in triplicate by the Kjeldhal method. Experimental precision was ± 0.02%. Stored protein was determined by the method of Barnshtein. Protein nitrogen was calculated by difference.

The yield and grain quality of the wheat in 1954 were formed under conditions of a sharp soil moisture deficit (Table 1). This was caused by a very dry spring and a prior drying out of the soil of the plot by perennial grasses.

When soil moisture was severely deficient, fertilization had little effect on increasing grain yield. Variety Gordeiforme 10 and Mil'turum 553 produced practically no yield increase and the yield of Lyutestsens 62 increased 1.1-1.24 centners/ha with the yield of the control 6.39 centners/ha. Dessicating conditions facilitate an appreciable leaf accumulation of total nitrogen, which in the long run is expressed as an increase in grain protein. The results of the 1954-1955 field experiments are shown in Tables 2 and 3.

It is evident from the data in Table 2 that there is a direct relationship between total nitrogen in the upper leaves during the flowering stage and total nitrogen of the grain. The nitrogen content of the leaves of Lyutestsens 62 was almost the same as that of the grain, in Gordeiforme 10 the correlation coefficient was 0.95, and in Mil'turum 553 it was 0.88 to 0.90 (Table 3).

During dry years a direct relation between the relative amount of leaf and grain nitrogen is also observed at the time of plant tillering. However, this relationship is closer during the flowering to milk stage.

The field experimental results obtained inspired us to verify whether the observed relationship between total nitrogen of the leaves and grain was maintained during growth of the wheat varieties under favorable con-

TABLE 5

Effect of Fertilizers on Yield and Nitrogen Content of Leaves and Grain of Spring Wheat

Treatment No.	Treatment No.	Grain yield	Grain protein, %	Grain	Total nitrogen, %	
					leaves	grain
	Mil'turum 553					
1	Unfertilized	26.8	13.74	368.2	3.06	2.61
2	$N_{30}P_{60}K_{30}$	32.1	11.57	371.4	2.37	2.23
3	$N_{60}P_{60}K_{30}$	34.4	13.45	462.7	2.75	2.58
4	Manure 10 m^3 + P_{60}	31.9	12.54	400.0	2.27	2.37
5	Manure 20 m^3 + P_{60}	34.9	12.0	418.8	2.25	2.30
	Lyutestsens 62					
1	Unfertilized	15.7	17.44	274.0	3.48	3.26
2	$N_{30}P_{60}K_{30}$	18.3	18.53	339.3	3.57	3.45
3	$N_{60}P_{60}K_{30}$	19.1	19.27	368.1	3.64	3.60

Note: Experimental error.

TABLE 6

Effect of Nutrition on Yield and Amount of Nitrogen in the Leaves and Grain of Spring Wheat

Treatment*	Dry wt. 12 plants, g/vessel	Grain	Oven-dry wt., g	Grain protein, %	Total nitrogen, %	
					leaves	grain
	Lyutestsens 62					
$N_1P_1K_2$	20.7	7.78	31.5	13.28	2.59	2.48
$N_2P_1K_2$	29.9	11.64	32.1	13.40	2.89	2.57
$N_3P_1K_2$	36.7	14.03	33.3	15.50	3.47	2.91
$N_2P_2K_2$	30.9	11.00	31.9	12.77	2.72	2.45
	Gordeiforme 10					
$N_1P_1K_2$	23.7	8.86	31.6	11.40	1.88	2.11
$N_2P_1K_2$	35.6	13.18	35.0	10.26	1.96	1.90
$N_3P_1K_2$	38.7	14.65	37.3	11.23	2.37	2.08
$N_2P_2K_2$	33.1	12.21	34.2	10.83	2.15	1.97

Experimental error 2.34—4.2 %.

* Numerical subscripts signify the amount of actual material per kilogram of oven-dry weight of soil (1 = 50 mg, 2 = 100 mg, 3 = 150 mg).

ditions of soil moisture and with high grain yield. In order to answer that question, a field experiment was conducted in 1956 and a greenhouse experiment in 1955-1956.

The field experiment was conducted with wheat varieties Lyutestsens 62 and Mil'turum 553. The varieties were sown at the same time in different plots of one field. The amount of winter snow and spring melt water was measured on the plot intended for the variety Mil'turum 553. The mineral fertilizers for this plot were prepared and applied in spring by plowing in to a depth of 14-16 cm. The mineral fertilizers were applied under the Lyutestsens 62 with a web-footed cultivator. The content of mobile nutrient materials before sowing on the control plots and the moisture trend in the sowed wheat varieties are shown in Table 4.

A difference in soil moisture and fertility and also a differing ability to incorporate fertilizers had a great effect on the effectiveness with which the different wheat varieties used fertilizers (Table 5).

TABLE 7

Effect of Increasing Rates of Nitrogen on Yield and Nitrogen Content of Leaves and Grain of Wheat Variety Lyutestsens 62 (1956)

Treatment No.	Treatment	Dry wt., 12 plants, g/ vessel	Wt. grain, g/ vessel	Oven-dry wt., g	Total nitrogen, g	
					leaves	grain
1	$N_2P_1K_2$	39.0	16.08	35.8	2.93	2.47
2	$N_4P_1K_2$	45.7	18.11	39.5	3.41	3.31
3	$N_6P_1K_2$	50.5	19.98	36.0	3.66	3.41
4	$N_4P_2K_2$	45.6	17.68	33.6	3.55	3.39
5	$N_2P_1K_2+N_2$	45.7	19.00	35.7	3.51	3.33
6	$N_2P_1K_2+N_2+N_2$	49.0	18.71	33.1	3.92	3.50

TABLE 8

Effects of Nutritive Elements on Yield and Grain Quality of Spring Wheat

Treatment	Dry wt., 12 plants, g/ vessel	Wt. grain g/ vessel	Oven-dry wt., g	Total nitrogen, %	
				leaves	grain
Gordeiforme 10					
Unfertilized	35.5	13.16	39.9	2.31	2.16
P_1K_2	39.2	13.80	40.6	2.08	2.11
N_3K_2	44.6	19.12	45.8	3.48	3.16
N_3P_1	53.5	21.06	40.2	2.96	2.75
$N_3P_1K_2$	60.5	21.09	37.8	2.72	2.56
Lyutestsens 62					
Unfertilized	31.8	12.80	31.5	2.54	2.41
$N_2P_1K_2$	55.3	22.23	32.6	2.85	2.47

A favorable water regime under variety Mil'turnum 553 and a soil nitrogen and phosphorous deficiency promoted a sharp response of the plants to applied fertilizers. A nitrogen rate of 30 kg per hectare in the NPK, by stimulating strong vegetative plant growth, appeared low for assuring a high grain quality. As a result grain protein in this treatment appeared lower than the control and the grass yield equaled that of the control treatment.

Increasing the nitrogen rate in NPK to 60 kg per hectare resulted in a grain yield increment of 7.6 centners / hectare with the grain protein almost equal to that of the control. Analysis of the top leaves during the flowering to milk stage, 30 days before harvest, showed that plants of the first, fourth and fifth treatments had a low percent of nitrogen in the leaves and thus, correspondingly, also in the grain. Therefore, leaf analysis data can be used for assessing plant need for nutritional nitrogen during the period from flowering to initiation of grain filling. The use of ordinary or nonroot nitrogen feeding during this period radically increases grain protein. [2, 11, 24-27].

Formation of yield and grain protein in the variety Lyutestsens 62 occurs with deficient moisture and significantly large amounts of mobile nutrient materials in the soil itself. Use of a complete mineral fertilizer with a nitrogen rate of 30 kilograms/ hectare increases grain yield 2.6 centners/ hectare. Increasing the nitrogen rate increases grain yield little, but markedly increases the percent protein in the grain. It is typical that in this variety leaf analysis gives a distinct picture of the absence of a plant nitrogen requirement and indicates the possibility of forming a grain with a correspondingly high nitrogen percent. Field experimental data affirms the connection and mutual dependence between the relative nitrogen content of the leaves and varietal growing conditions, on the one hand, and the relative nitrogen content of the leaves and the grain on the other. This dependence was also confirmed in the greenhouse experiment in 1955.

Soil for the greenhouse experiment in 1955 was taken from a long-ploughed field on which potatoes had been raised during the previous two years. One kilogram of oven-dry soil contained 12.5 mg of NO_3-N, 136.4 mg P_2O_5 (by Truog), 101.4 mg of K_2O (by Brovkin).

Experimental results are presented in Table 6.

From Table 6 it is evident that increasing the rate of nitrogen increases the yield and oven-dry weight of the grain. The two varieties do not react to an increase in the phosphoric acid rate. It is typical that variety Lyutestsens 62 by creating a smaller vegetative mass, better utilized the mineral fertilizers for increasing grain

TABLE 9

Effect of Nitrogen Added at the End of Flowering Upon the Nitrogen Content of Lyutestsens 62 Spring Wheat

Treatment*	Grain yield g/vessel	Leaf nitrogen, % dry wt		Grain nitrogen, % dry wt	
		end tillering	end flowering	Forecast, ±, 1%	Actual
Control	3.26	2.13	2.02	2.02	2.56
$P_{50}K_{100}$	3.25	2.09	2.08	2.08	2.75
$N_{50}P_{50}K_{100}$	6.81	3.17	2.06	2.06	2.13
$N_{100}P_{50}K_{100}$	10.71	3.66	2.48	2.38	2.31
$N_{20}P_{50}K_{100}$	4.64			2.20	2.31
$N_{20}P_{50}K_{100} + N_{80}$ (Root fed)	4.22			–	3.85
$N_{20}P_{50}K_{100} + N_{80}$ (nonroot fed, soil not isolated)	4.30	2.71	2.20	–	3.44
$N_{20}P_{50}K_{100} + N_{80}$ (nonroot fed, soil isolated)	4.19			–	3.42

* Subscripts signify the rate of the nutrient materials in mg/kg oven-dry soil. Soil moisture was 80% of field capacity. Experimental error 1.8-4.1%.

protein. The second reason for the increased amount of protein in the grain of this variety is linked with its rapidity of maturity [1, 19]. The season of variety Lyutestsens 62 is 73 and of variety Gordeiforme 90-94 days. The formation and filling of the grain in Lyutestsens 62 occurs at respectively lower (65%) and in Gordeiforme 553 at higher (72%) relative humidity. A higher grain protein in Lyutestsens 62 can be foreseen by leaf analysis six days after completion of plant tillering (July 7), i.e. a month before harvest. Leaf analysis of Gordeiforme 10 was made at the termination of the flowering to milk stage (July 28) but the $N_1P_1K_2$ treatment already had normal sized grain. Apparently, the departure of this treatment from the general rule is explained by this. The data on total nitrogen of the leaves and grain are closely similar and well reflect the varietal differences of wheat in forming grain of a certain quality [19, 21]. The higher percent of nitrogen in the leaves, the greater the relative nitrogen and protein content of the wheat grain. This confirms the results of Darkanbaev [3] that the condition of the plant itself, which determined the tempo of its growth stages, is closely linked with external environmental conditions, and exerts a decisive influence on the trend of accumulation of materials, among them the amount of protein nitrogen in the grain.

The effect of increasing rates of nitrogen on the yield and quality of the grain of Lyutestsens 62 was tested in a greenhouse experiment during 1956.

Soil for the experiment was taken from the same plot as for the greenhouse experiment in 1955. In one kilogram of oven-dry soil was found 14.4 mg of NO_3-N, 177.3 mg P_2O_5 (by Truog) and 122.0 mg K_2O. Soil moisture approached 60% field capacity. Experimental results are presented in Table 7.

From the data of Table 7 it is apparent that on soil which is poor in nitrogen increasing the rate of nitrogen to 300 mg/kg of soil (N_6) increases the yield and improves the quality of wheat grain. Variety Lyutestsens 62 both in 1955 and 1956 did not respond to a double rate of phosphorous because of the presence of sufficient soil P_2O_5. Transference of part of the nutritional nitrogen to the three-leaf stage (treatment 5) and during the three-leaf and five-leaf stage (treatment 6) did not increase the grain yield above application of the entire nitrogen dose before sowing, but augmented the percent of nitrogen in the wheat leaves and grain. A direct relation between leaf and grain nitrogen with a very high coefficient of correlation was also found in this experiment.

The soil of field plot No. 2 was used in another greenhouse experiment (see Table 4).

The effect of individual nutritional elements upon yield and the nitrogen content of leaves and grain of variety Gordeiforme 10 was tested. In addition, for comparison with the preceeding greenhouse experiment

(Table 7), an experiment with variety Lyutestsens 62 was laid out on the more fertile soil of a second area. The experimental results are presented in Table 8.

The data in Table 8 have a greater uniformity and show that application of nitrogen fertilizers increase the amount of nitrogen in the leaves and, correspondingly, in the grain of spring wheat. The effect of nutrient conditions upon the amount of nitrogenous materials in the grain of wheat is reflected in the accumulation and translocation of nitrogenous materials in the leaves and the entire plant during the process of its complex cycle of development [3, 19].

Our experimental data permit us to come to a conclusion concerning the direct relationship between the total nitrogen of the four top leaves during the flowering to milk stage and the total grain nitrogen during the full ripening period. The ratio between these two values is very stable and with normal, or close to normal, conditions of nitrogen nutrition for spring wheat it is 0.96 for Lyutestsens 62, 0.93 for Gordeiforme, and 0.90 for Mil'turum. According to our experimental data a normal level of nitrogen and phosphorous nutrition corresponds to 2.6 – 3.0 percent nitrogen and 0.52-0.60 percent P_2O_5 in the leaves at the end of the flowering stage.*

Such a concentration of nutrient materials in the leaves assures, under favorable conditions, a high grain yield of good quality.

With a decrease in leaf nitrogen content from 2.5 to 2.0%, when the plants have a good supply of soil moisture before flowering and because of an average (15-20 centners/ha) or high (25-35 centners/ha) grain yield, the coefficient of transfer of nitrogen from the leaves into the grain is independent of varietal peculiarities (cf. Tables 5, 6, 9). Under these conditions plants receive insufficient nitrogen for the formation of high protein grains and supplementary nitrogen fertilization at the end of flowering is essential (Table 9).

A decrease of leaf nitrogen below 2% assures the appearance of externally visible signs of plant nitrogen deficiency and, in view of the low grain-yield, it is a strong indication of plant nitrogen requirement on a given soil. Under these conditions feeding during the flowering stage is not expedient since with a low grain-yield the nitrogen content is frequently high [2]. A direct relationship between total leaf and grain nitrogen under severe nitrogen deficiency conditions is confused during the tillering stage [21b].

The need for using leaf analysis data to establish plant nitrogen requirement and for various approaches to nitrogen application during the flowering stage was confirmed by experiments in 1957. Two varieties of spring wheat were raised in a greenhouse experiment. Plants were analyzed during the three leaf stage, at the end of tillering, and during the flowering to milk stage.

The analysis showed that in the first two treatments the leaves had a lower percent nitrogen during these stages and a lower grain yield (Table 9). Therefore, the evaluation of grain quality in this treatment was difficult during the flowering stage. Analysis of wheat leaves from the $N_{20}P_{80}K_{100}$ treatment for nitrogen during the flowering to milk stage prognosticated a low grain-nitrogen content. Three vessels of this treatment were retained as controls and on the day after the analysis nine vessels receive one application of NH_4NO_3 (0.9% solution) and two applications of urea (0.7% solution). Feeding was conducted for a day, with 50 ml of solution per vessel by the root and extraroot method (with and without the soil isolated). Experimental results on variety Lyutestsens 62 are shown in Table 9. The data of this table indicate the great effectiveness of nitrogen additions at the end of flowering in raising the nitrogen percentage in spring wheat grains. Such an addition is one of the management practices which can raise the protein content of wheat in regions in which this crop has a low grain quality [15, 27].

Greenhouse and field experimental data in 1957 confirm the possibility of a quite exact forecast (± 0.1% nitrogen) of grain quality in spring wheat, and leaf analysis furnishes the basis for nitrogen application during the flowering to milk stage. In this regard, chemical analysis of the leaves, which exert a very great effect on formation and filling of the wheat grain, and the establishment of a coefficient of correlation between the chemical status of the leaves and the grain, apparently, is a more correct method for diagonosis of grain quality [2, 3, 21] in contrast to the establishment of a connection between the amount of precipitation and grain nitrogen, passing through the plant [28].

* A more exact level of nitrogen nutrition for spring wheat will be specified in another work.

RESULTS

1. A connection and mutual dependence was shown between leaf nitrogen content and plant nutrition conditions on the one hand, and the relative amount of nitrogen in the leaves and grain on the other.

2. A direct relation, with a very high coefficient of correlation (0.90-1.0), has been established between the nitrogen content of the top leaves of wheat during the flowering-milk stage and grain nitrogen at full maturity.

3. On the basis of leaf analysis during the flowering stage it is possible to forecast grain quality, to establish the plant nitrogen requirement and to determine the need for applying nitrogen fertilizer in the later stages of growth and development of the wheat.

4. Root and nonroot nitrogen feeding at the end of flowering sharply increases the percent nitrogen in the grain of spring wheat.

SUMMARY

An important chemical characteristic of the quality of wheat grain is its nitrogen content.

The purpose of the present investigation was to study how the presence of nitrogen in the upper vegetative leaves of the spring wheat plant during flowering influences the relative content of nitrogen in the grain of wheat. This stage was chosen because by that time the plants on a whole stop accumulating nitrogen, growth of leaves and stems has terminated, and the transport of substances from the organs into the seeds has begun.

Four upper leaves, counting from the spike, were studied. Ten experiments were performed with three types of wheat. The experiments were carried out under field conditions and by the vegetative vessel method during 1954-1957. The following was established.

1. A connection and interdependence exists between the relative content of nitrogen in leaves and the conditions of plant nutrition, on the one hand, and the relative content of nitrogen in the leaves and nitrogen content in the grain, on the other hand.

2. A positive correlation has been established between the relative content of nitrogen in the upper leaves of the wheat plant at flowering and the nitrogen content of the grain at full maturity.

3. The quality of grain can be predetermined long before harvesting by analysing the nitrogen content in the leaves.

The coefficient for reduction of total nitrogen in leaves to nitrogen content in the grain is 0.96 for Lyutestens 62, 0.93 for Gordeiforme 10, 0.90 for Mil'turum 553. The error in forcasting the amount of nitrogen in grain does not exceed 0.08% of the total nitrogen.

4. A study of the leaves offers a means of determining the requirements of plants for nitrogen, and the necessity of nitrogen fertilizers at late periods of growth and development.

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BRIEF COMMUNICATIONS

THE APPEARANCE OF BRANCHED SPIKES IN COMMON VARIETIES OF CEREAL CROPS

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Branched spikes in cereal varieties, which have been in existence for a century [1], have recently attracted the attention of investigators as being forms with exceedingly good prospects for agricultural use, since they posses a number of valuable anatomical and morphological features [2] and the ability to produce a large amount of seed.

Study on the question of the origin of branched spikes shows, that favorable plant nutrition is an indispensable condition for the formation of these spikes. Moreover, certain additional methods have been employed by various workers: sexual and vegetative hybridization [3-5], growing plants with varying day lengths [6, 8], vernalizing at various temperatures [6, 9, 10] and certain others. A majority of the authors assumed that variation in the tempo of growth and development is a decisive condition for the formation of branched spikes.

Experiments which we have conducted showed that the fundamental distinction between the formation of a branched and a simple spike lies in that the lateral growing points of a branched spike arise not only in the axils of the second pair of leaf primordia, but also in the axils of the first pair. Moreover, a growing point originating here does not flower, but forms spike tubercles (second order) since second order spikes develop from them. If plants of branched rye grow in a condition of deficient nutrition, they may form ordinary unbranched spikes. In this event the growing point of a first order spike converts to flowering. Consequently, this growing point can function as a spike or a flowering lateral, depending on the conditions. It could be also supposed that among the ordinary spikes of rye variety Vyatka a spiklet has the same such potential, however, during normal development, because of a hereditarily fixed order of distribution of materials which determine the tempo of growth of the organ, this potential of a spike is completely unrealized. The branched variety in this respect is more plastic. It might be supposed that an increase of plasticity is a fundamental characteristic peculiarity of cereal varieties with branched spikes. It follows from this that the first undertaking in conversion of ordinary into branched types is to attain a hereditarily high basic plant plasticity. The second task is to select the conditions which cause the features that interest us to appear. It is completely logical to suppose that one such a condition is a copious supply of plant mutrient materials.

In order to overcome the heriditary conservationism of the plant we employed in our experiment the method developed by T. D. Lysenko, that of stepped up temperatures at the end of the vernalization stage. Conditions of copious nutrition were created by split application of increasing rates of mineral fertilizers. It was determined by a special experiment that for the object of the experiment 40 days was the most favorable duration of presowing vernalization. The necessary nutritional regimen and fertilizer rate was ascertained by another series of experiments. To every kilogram of oven-dry soil in the greenhouse vessels was added: 0.3 g NH_4NO_3, 0.175 g NaH_2PO_4, and 0.236 g KCl before sowing; 1.8 g NH_4NO_3, 0.525 g NaH_2PO_4, and 0.708 g KCl in the first feeding (tillering stage); 1.2 g NH_4NO_3, 0.350 g NaH_2PO_4, and 0.472 g KCl in the flower formation stage.

There were four experimental treatments: 1) 50 day vernalization, without fertilizer, 2) 40 day vernalization, without fertilizer, 3) 50 day vernalization, copious fertilizer, and 4) 40 day vernalization, copious fertilizer.

Plants were raised in clay jars with about 5.3 kg of oven-dry soil and with five plants per jar. Each treatment contained 20 vessels: 10 vessels remained until plant maturity and the other 10 vessels were used for morphological analysis. In addition to this, part of the seeds from both vernalization periods were sown out in soil beds fertilized well with manure and mineral fertilizers. In addition feeding was carried out as in the greenhouse experiments.

EXPERIMENTAL RESULTS

Analysis of the spikes from the sowing in the bed showed that, among the unvernalized plants occupying an area of one square meter, several of the clumps had truly branched spikes (Fig. 1). There was also an appreciable number of spikes with up to 4-5 subinflorescence per spike. In a second plot, where completely vernalized seeds had been sown, not one altered spike was found.

Branched spikes and ones with many inflorescences were found only in the fourth treatment of the greenhouse experiment. Plants vernalized for 50 days did not form even one branched spike or one with many inflorescences. Also, there were no such spikes in the second treatment.

A morphological examination of the embryonic spikes revealed certain regularities in the transformation of an ordinary spike into a branched one. It was noticed that only a spike having a large number of lateral growing points (more than 9) could branch. In a second-order spikelet being transformed, only the terminal growing points and two or three below them, always form inflorescences. Therefore, on the axis of a branching first-order spike there are two completely developed inflorescences below and a second-order spikelet above (Fig. 2, E). Sometimes, that part of the first-order spike located above the inflorescences at the time of spike emergence dies together with the second-order spikes situated on it, and the spike becomes unbranched for the second time.

Fig. 1. Branched spikes of rye variety Vyatka obtained in 1954 experiments.

The very early stages in transformation of an ordinary spike into branched one are depicted in Figure 2. The first-order spikelet (2, E) has two spike bracts and two normally developed florets below, and the remaining growing points are appreciably retarded in development. They remained in a state of dormancy for a certain time, but under the influence of the experimental conditions they emerged from this condition and the processes of growth and development were regenerated in them. Although from the external appearance of lateral growing points number 5 and 6 it might be concluded that not florets but secondary spikelets are being formed from them, since with their formation the growth of the central growing point (pistillate protuberance) is markedly inhibited, compared to that of the laterals from which staminate are being formed, the reverse was observed here. Particular attention is merited by growing point four, from which is formed the first second-order spikelet. The lateral growing points of this spikelet are likewise laid out with three staminate protuberances in a circle. This indicates that the transformation of a floral protuberance into a spikelet began at a time when the staminate protruberances had already been laid down and that later, at certain times during ontogeny, they can be converted into flowers similar to the conversion of an inflorescence protuberance into a spike. That spikelets of the second-order are formed from first-order floral protuberences also indicates the presence of a "floral bract" in certain second-order spikelets during one or another stage of development, which appears here as a leaf enclosing the spikelet. (Fig. 2, B, C, D). Hence, the second-order spikelets develop from dormant growing points when, for some reason, the resting stage is disturbed.

There are indications in the literature that dormant buds are aroused from rest when a certain store of nutrient substances is accumulated at their location [11]. The level of mineral nutrition created in our experiment, could completely assure the accumulation of a large amount of organic material. However, during the normal course of the vernalization stage no branched spikes were formed and, subsequently, neither did the resting growing points break dormancy with a copious supply of plant nutrient materials present. This means that completion of the vernalization stage at an elevated temperature has a substantial role in this process. As we know, the duration of the vernalization stage is appreciably increased and the total period of plant vegetative growth is lengthened. However, this can not have much influence, since the process of secondary spike formation occurs long after than the vernalization stage when the normal relationship between growth and development is reestablished.

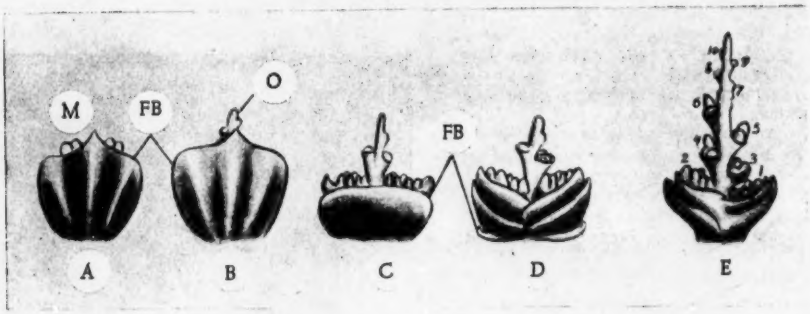

Fig. 2. Conversion of an ordinary spike of rye variety Vyatka into a branched one. A — Normal inflorescence (side view of the lower tract of the inflorescence). B, C, D — second-order spikes developed from rudimentary inflorescences and retaining to varying degrees the development of the inflorescence bract; E — early stage in conversion of floral protruberences into second-order spikes; FB — lower tract of the inflorescence; O — axis of spike; 1, 2, 3 — florets on the first-order spike; 4, 5, 6 — second-order spike; 7, 8, 9, 10 — terminal spikelets of the first-order spike.

Don't unusual meterological conditions exert an effect on the process of spike formation, in that a lag in going through the vernalization stage causes a shift to a period much later than usual (June-July)? In any one of the late sowings of seeds of the rye variety Vyatka which had completed vernalization before sowing, spike formation occurred during the same period but no aberration in the form of the spikes was observed. Therefore, it must be suggested that completion of the vernalization stage at a stepped-up temperature interrupts the usual course of metabolism, renders the organism more plastic and vital and alters the natural, ancient, inherited basis for the order in which nutrient materials are apportioned between various growing points. Such changes in the organism, in conjunction with the presence of a sufficient supply of essential nutrient materials in the surrounding environment, can not only rouse a resting growing point from dormancy but can also assure an acceleration in the growth of it afterward. With such a combination of internal and external conditions there occurs a more complete realization of the potential possibilities for a spike and spikelet to branch.

The lower floral protuberances, as we have already mentioned above, sometimes do not change into spikelets of the second-order. This can be explained by the fact that the plant assimlatory apparatus still does not produce enough nutrient materials at the time that the lower inflorescences are forming and, as a result, the protuberances lose the chance to branch because a new epoch or stage of their development begins. During the dormant period the top growing points retain this possibility until they break dormancy, and it is realized under the influence of a copious inflow of nutrient materials manufactured in the vigorous fully-formed organs.

From what has been said above, it becomes evident that in our experiments spike branching occurs from the combined effects of a number of factors, among which a copious supply of mineral salts and water to the plant plays a fundamental role. We are not inclined to think that these materials have some sort of specific value for the generation of branched spikes and suggest that they are effective by virtue of the existing conditions under which they can facilitate an increase in the amount of organic material in the plant. Any other

factor able to increase the concentration of organic materials in the plant to a certain level will also be able to cause branching of the spike.

The unstable nature of the plant investigated ought to be considered as a second very important factor which conditions branching of a spike. However, inherited instability is only an accessory factor on the basis that an excess of nutrient materials appears to be the basic factor with which it closely interacts.

Seeds obtained from branched spikes and ones with multiple florets were sown out on the ground during a different year under natural vernalization conditions. In the yield obtained only individual spikes had two spikelets, most of the spikes had multiple inflorescences. There were five to six or even eight inflorescences per spike. There were also a few branched spikes. On the basis of this it can be hypothesized that with the creation of more favorable growth conditions for these plants, the branching characteristic will be inherited in the descendants.

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CONCENTRATION OF LEAF CELL-SAP AS A PHYSIOLOGICAL INDEX OF WATER SUPPLY IN THE COTTON PLANT

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A method has been worked out by Labov [1] for determining the time of irrigation of vegetable crops and potatoes,on the basis of leaf cell-sap concentration measured with a refractometer. The simplicity and practicality of a determination of cell-sap concentration is an advantage of this method. Our use of the refractometer method for determination of leaf cell-sap concentration confirms the possibility that this index can be used for assessing the status of the water regime of cotton [2]. The concentration of the cell sap of a certain leaf-story of cotton, usually the growing point, characterizes the status of the plant water regime. With the objective of thoroughly evaluating this index,we have conducted supplementary investigations by studying the effect of various factors in the magnitude of cotton leaf cell-sap concentration and by refining the critical value which signals the need for irrigation.

The investigations were carried out on the experimental fields of the Ukranian Scientific Research Institute of Irrigation Agriculture (Kherson), where cotton had been cultivated earlier. Regular determinations of cell-sap concentration in various experimental treatments were carried on through 1954, 1955, and 1956.

Three to four irrigations were applied in the experimental treatments during the vegetative stage. Cotton fiber yield reached 20-22 centners per hectare with 14-16 centners per hectare before frost.

Yields reached 4-6 centners per hectare in the unirrigated treatments. Variety 611-b was sown.

The soils on the plots are chestnuts, low in organic matter, weakly solonetzic, and mechanically an easily friable clay. Two to three centners of ammonium sulfate and superphosphate per hectare were added to the experimental plots.

Cell-sap concentrations were determined at 1-2 P.M. every five days beginning with the budding stage. Six to eight morphologically mature leaves, usually the fourth from the growing point, were selected from each treatment for analysis. Drops of the sap squeezed from leaf pieces were poured onto the prism of the refractometer. Cell-sap concentration is expressed in conventional values,which characterize the optical activity of a sucrose solution at one concentration or another,to which optical activity of the cell-sap is equivalent. In 1955 and 1956 temperature and humidity of the air was determined simultaneously with an Assman psychrometer. In addition, every ten days soil moisture was determined to a depth of one meter from tube samples taken every ten centimeters. Soil moisture determinations were replicated six times.

The results of three years of experiments show that the cell sap concentration of cotton leaves systematically varied in relation to soil moisture. With high soil moisture,which occurred during the first days after irrigation or with an abundant rain, the leaf cell-sap concentration was very low. It was always lower on the irrigated plots than on the unirrigated. The cell sap concentration of the cotton leaves increased gradually in proportion to the drying-out of the soil. Under conditions of abundant soil moisture the cell sap concentration of leaves fourth from the growing point never exceeded 11-12%, and with soil drought reached 20-22% or more. We are presenting data from the 1955 season (Figure) to illustrate this. The curve for the cell-sap concentration of the leaves to an appreciable degree is a mirror image of the curve for soil moisture change. Meanwhile, the amplitude of temperature changes and air humidity at the time determinations were made did not have a mater-

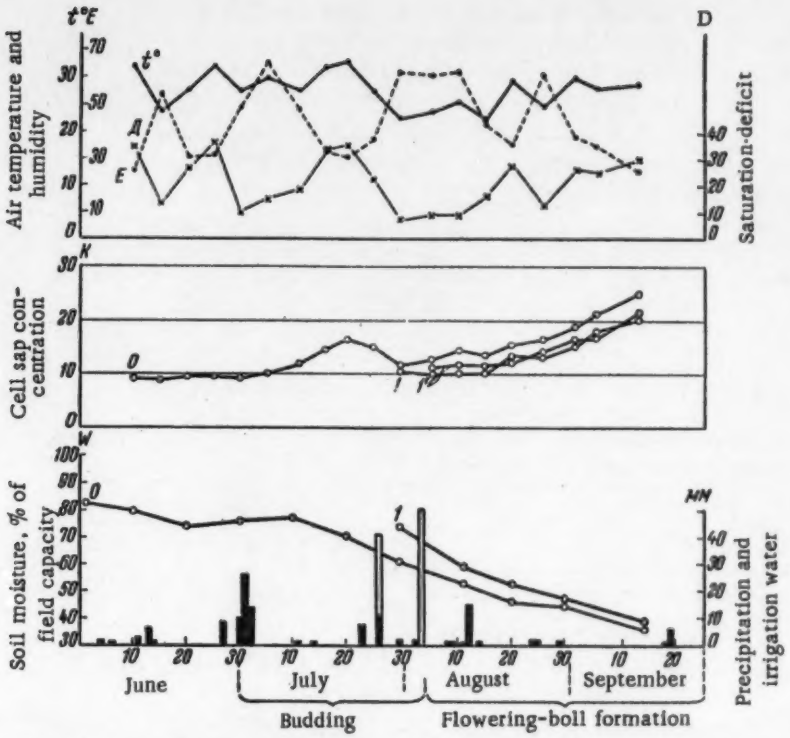

Fig. Influence of external environmental factors on the cell-sap concentration of cotton leaves.
O — Unirrigated; 1 — irrigated 26 July; 1 — irrigated 3 August; t° — air temperature, °C; E — air relative humidity, %; D — air saturation deficit, mb. Black columns — precipitation; light columns — irrigation water (soil moisture in the irrigated treatments was nearly uniform and has been represented by one of them).

ial effect on the cell-sap concentration of the leaves. The curves and data obtained in 1956 were of an analogous character.

In Table 1 are presented data from a statistical evaluation of the effect of various factors on the magnitude of the cell-sap concentration of leaves.

A high and steady positive correlation exists only between the cell-sap concentration of the leaves and soil moisture. Any effect of temperature and humidity on cell sap concentration was insignificant, or completely absent. Consequently, soil moisture is the basic factor determining the magnitude of cell-sap concentration of cotton leaves.

An effect of temperature and humidity can be observed only with an abrupt departure of weather conditions from normal. Thus, on a hot day with a dry wind or on a cloudy day the cell sap concentration of the leaves is 2-3% higher or lower than usual. Diurnal variations in cell-sap concentration follow the course of changes in weather conditions and usually are also 2-3%. Only when the soil is dry does the amplitude of these changes increase.

In addition to the foregoing analysis we also indicate a high correlation between cell-sap concentration and leaf DPD which was determined at the same time. The coefficient of correlation between them in 1955 was (+0.85) and in 1956 (+0.71).

TABLE 1

Coefficient Correlation Between Cell-Sap Concentration of Cotton Leaves (K), Soil Moisture (W), Temperature (t°), Air Absolute Humidity (A), Relative (E), and Saturation Deficit (D)

Factors compared	Correlation coefficients	
	1955	1956
K W	-0.85 ± 0.058	-0.83 ± 0.060
K $t°$	$+0.15 \pm 0.122$	$+0.16 \pm 0.163$
K A	-0.31 ± 0.113	-0.29 ± 0.152
K E	-0.35 ± 0.110	-0.03 ± 0.166
K D	$+0.23 \pm 0.120$	$+0.37 \pm 0.143$

TABLE 2

Cell-Sap Concentration of Cotton Leaves at the End of the Vegetative Period in Relation to Soil Moisture, 1956

Soil Moisture	Date Determined			
	9/5	9/10	9/15	9/20
Normal with two seasonal irrigations	18.4	16.5	15.7	15.4
Copious at the beginning of the irrigation furrow	13.0	11.7	11.5	13.3

Thus, the cell-sap concentration of the leaves is an objective and reliable index of the state of water regime of cotton. The value of the cell-sap concentration determined on the fourth leaves from the growing point with plentiful soil moisture remains at a low level during the whole vegetative period. As we have shown earlier [2] this assures an intensive stem growth and the gradual formation of a zone of young developing leaves typified by a low cell-sap concentration. The data is Table 2 support this.

The cell-sap concentration of leaves of typical plants in the treatment with two seasonal irrigations reached, at the beginning of September, 18.4% and gradually declined to 15.4% after the rains which fell on the eighth and ninth of September. Cell-sap concentration in plants of the copiously moist plot (at the beginning of the irrigation furrows) did not exceed 13.3% during the vegetative period. These plants reached a height of 120 cm. Meanwhile, in 1955, the cell-sap concentration of the fourth leaves from the growing point had risen to 16% during the budding stage before the first irrigation.

On the basis of three years of investigation we have come to the conclusion that the critical value of the cell-sap concentration of cotton leaves determined on the fourth leaves from the growing point during the period from budding to the beginning of ripening is constant. With a cell-sap concentration of 12% or less in the fourth leaves from the growing point, the plants typically grow rapidly and have normal leaf turgor during the whole day. Maintaining such a water regime during the entire vegetative period can cause over growth (oiliness) of cotton. An increase of the leaf cell-sap concentration in 13-14 days to 13-14% indicates the onset of brief plant water deficit during the daytime hours. Such a water regime assures a more formable ratio between cotton growth and development, facilitating the obtaining of a higher yield of cotton fiber of good quality. The beginning of soil drought is characterized by a rise in the leaf cell-sap concentration to 15-16% accompanied by wilting of the leaves during the daytime hours. With severe wilting the leaf cell-sap concentration rises to 20-22% or more. This regularity is clearly expressed with timely scheduling of irrigations and sufficient penetration of soil moisture to 60-70 cm. In some cases we noticed departures from this rule. With frequent light irrigations (300-400 m^3/hectare), plants which were growing rapidly may quickly show a sudden water deficit. The leaves begin to wilt with a much lower cell-sap concentration in the leaves to the extent of 13-14%. On the other hand, with excessively delayed irrigations the cell-sap concentration of the leaves falls gradually after irrigation. The plants, not able to sustain normal physiological functions, quickly use up the stored mois-

ture, especially with light irrigations. Therefore the concentration of the leaf cell-sap, with normal turgor, can fall only to 15-16%. Moreoever, plants which experience a water deficit for a protracted period adjust to soil drought condition by discarding the lower leaves. The concentration of the cell-sap of the leaves remaining on the plant increases to 18-20% and to normal turgor, which we observed, on unirrigated treatments at the end of the vegetative period. However, these departures do not exclude the possibility of diagnosing the irrigation time for cotton on the basis of the cell-sap concentration of the leaves, in correspondence with biological requirements of the plants at the time of each irrigation.

The refractometer method for determination of the concentration of the leaf cell-sap is simple and practical, particularly when a field refractometer is used. With its use good results are usually obtained from determinations on a drop of sap squeezed from a leaf freshly stripped from the plant. In those cases where the visibility of the divisions in the eyepiece of the refractometer is poor, which occurs with abundant nitrogen nutrition, it is expedient to insert the leaf rolled up in gauze into an aluminum thimble and heat it over an alcohol flame or in a desiccator, thus avoiding an over-heated sample. It is easy to squeeze a large amount of cell-sap from the tissues of minced leaves, which gives a clear reading in the eyepiece of the refractometer.

In presenting the results of these investigations it is necessary to emphasize the need for verification and refinement of the critical values for the concentration of cell-sap in cotton in relation to the edaphoclimatic conditions of the principal cotton planting districts and also in relation to cotton variety.

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IRON AND MANGANESE IN PHOTOSYNTHESIS

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Through the use of tagged atoms in the study of photosynthesis, it has been possible to show that, in a plant cell, the divergence in pathway of the carbon from reduced carbon dioxide from that of the oxygen separated occurs during a very early stage in the process and is accomplished, apparently, with the participation of a system of catalysts. In one of the hypothesized schemes for photosynthesis this divergence is presented in the following form:

$$CO_2 \cdot X \cdot Y + H_2O \xrightarrow{\text{light}} XH \cdot (CO_2) + YOH \qquad (1)$$

According to this scheme, the reduction of carbon dioxide occurs in connection with the substance X, the separation of oxygen depends upon substance Y. This stage in the formation of initial products is very important for the path of the whole process, since here the opportunity for reverse oxidation of the obtained products by the oxygen evolved is avoided. Despite differing hypotheses as to the possible nature of materials X and Y, there has been no direct data on this until recently. From our knowledge of the elementary composition of plants, it was naturally assumed that metallorganic substances entered into the makeup of these catalysts, participating in the creation of an oxidation-reduction system within the plant [2]. At the present time it is known that a large number of metals enter the makeup of various oxidation-reduction enzymes, and the number of them has increased in recent years [3].

During a study of the pathway of carbon in photosynthesis the authors of this paper were able to find iron in the makeup of the initial products of $C^{14}O_2^-$ reduction [4, 5]. Tagged carbon from carbon dioxide very rapidly changed into a polyoxy acid with a molecular weight of about 1500 and containing 4% iron. A subsequent reduction, with the conversion of C^{14} from a carboxy into an alcohol group, also occurs in this substance which thus appears as a complex XH. (CO_2).

With regard to the nature of the catalyst Y it was hypothesized that it might be a manganese compound which took part in the formation of photosynthetic peroxides. The addition of manganese salts to a suspension of illuminated chloroplasts increases the oxidation – reduction potential [6]. That the first step of the photosynthesis reaction connected with oxygen evolution is suppressed in algae when manganese is absent from the medium appears to be further support for this view [7].

In order to explain the significance of them in photosynthesis, we made determinations of iron and manganese in whole leaves of Primula obconica and Trifolium repens, and also isolated from these materials fractions containing the first products of the photosynthetic reduction of carbon dioxide. After four-hour experiments in light at 5000 lux at a temperature of 25° or in darkness, the leaves were chopped up in an isotonic sucrose solution at 1-3°, the extract was filtered through a glass filter, and was precipitated in 60% acetone. The precipitate was further washed with cold 100% unacidified acetone and then water. After this treatment there remained an insoluble fraction in which nearly all of the C^{14} is found during photosynthesis, about 1-3% of the leaf dry weight. When it is dissolved in a 0.1N HCl, the part labially linked with the tagged C^{14} carbon dioxide is released. Neutralization of the solution to a slightly acid reaction, about pH 5, may precipitate from it the iron-containing polyoxy acid which seems to be the initial product of the assimilation of $C^{14}O_2$ during photosynthesis. An appreciable amount of Mn remains in solution here. Part of the Fe and Mn compounds

TABLE

Iron and Manganese in the Initial Products of Photosynthesis

Plant History	% Fe in Leaf Polyoxy acids		% Mn in Leaf Flavoproteins	
	HCl soluble	HCl insoluble	HCl soluble	HCl insoluble
Primula				
Darkness	0.014	0.019	0.028	0.010
Photosynthesis	0.045	0.013	0.012	0.020
Light + 10^{-2} M NH_2OH	0.017	0.025	0.023	0.008
Trifolium				
Darkness	0.005	0.006	0.008	0.003
Photosynthesis	0.012	0.005	0.003	0.006

does not transfer into a hydrochloric acid solution. These may be separated after a few minutes of boiling with water and precipitation of the iron-containing polyoxy acids in the extract with barium, the manganese compounds remaining in solution.

By fractionation of incinerated materials which had transfered into the 0.1N HCl and those insoluble in it, we found that in the first case the Fe in the oxyacid was bivalent and in the second case trivalent. Iron determinations were conducted in parallel, by both the rhodanine and dipyridyl method [8]. Manganese in the hydrochloric acid extract was divalent only, and manganese at the higher degrees of oxidation remained in the insoluble precipitate. In the beginning of the work, total manganese was determined by oxidation to permanganate by the method of Lavrukhina [9], and later was determined by the method of Wiese and Johnson [8, 10] by oxidation of the manganese with sodium bismuthate, a method suitable for work with small amounts of material. In order to establish that there was a difference in valence state before incineration, it was necessary to identify iron directly in the polyoxy acid (with rhodanine or dipyridyl) and manganese in its compounds (by reaction with benzidine under acid and alkaline conditions, etc.) [11, 12]. These experiments affirmed the highly developed regularity in distribution of the Fe and Mn compounds. It is interesting to see that, coincident with an increase of reduced iron, there occurs an increase of oxidized manganese (see Table).

Previous determinations of the percent distribution of C^{14} in an polyoxy acid molecule showed that the reduction of the C^{14} occurs here, only part of the C^{14} remaining in the carboxy groups, the remaining percent of it being found in the alcohol groups [5]. The ease of reducing the carboxy group into an alcohol has been described for iron-organic compounds of the ferrosine type [13]. The amount of manganese in the initial products fraction in relation to the dry weight of the leaves was close to that of iron found here, about 0.01-0.03%. In contrast to iron, almost all of the manganese was found in this fraction of the leaves. As was indicated above, when the iron-containing polyoxy acids are precipitated, the manganese remains in solution but may precipate after neutralization. In this way we obtained preparations containing about 1% manganese. This organic compound belongs to the flavoproteins, but the metal is comparatively easily separated. Probably, a rapid removal of oxygen from the products of carbon dioxide reduction is achieved by oxidation of this compound in light.

Photosynthesis ceased after the introduction of the enzyme poison hydroxylamine at a concentration of 10^{-2}M into the leaf by the vacuum filtration method of Kursanov, and with subsequent illumination of the leaves the tagged carbon from carbon dioxide was found only in the initial product, the polyoxy acid, not more than 0.01% of the leaf dry weight. Under these conditions manganese oxidation did not occur: the relationship was the same in both light and darkness. This is as if the paths of $XH \cdot CO_2$ and YOH separation were blocked. The presence of these catalysts Fe and Mn and the characteristic effect of enzyme poisons on them affirm the probability that here the hydrogenation system participates by carrying out various reactions at a low value of the oxidation-reduction potential [3, 4].

The oxidation-reduction potential found indicates a new possibility for studying the chemistry of photosynthesis, and also the link between it and plant mineral nutrition.

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THE EFFECT OF OXYGEN ON SPECTRUM CHANGES INDUCED BY LIGHT IN PLANT LEAVES

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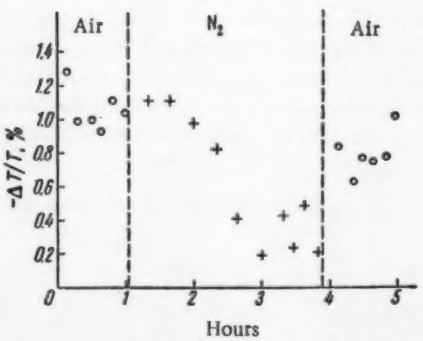
As shown by many investigators, the exposure of algae or leaves of the higher plants to red light results in changes in the spectrum passed through these objects. At the present time the most important of the problems is the examination of the nature of the process which causes these spectrum changes. The examination of the conditions of either the final oxidation or deoxidation of the product which changes the spectrum observed in the experiment is especially interesting. With this as our goal, we changed the oxidation-deoxidation conditions in plant cells by the periodic removal or introduction of oxygen in the atmosphere surrounding the leaves.

The experiment was carried out with the newly emerged leaves of young plants of horse beans. The leaves were enclosed in flat airtight boxes provided with two outlets for passing gases throughout the box. The middle portion of the box was covered with clear glass on both sides for observation of the leaves.

The changes associated with $\lambda = 515m\mu$ in a spectrum analysis induced by red light were measured on the differential spectrophotometer [1] for the passage of normal air through the box and for the passage of nitrogen or nitrogen enriched with carbon dioxide (5% CO_2).



The diagram shows the results of one of the experiments. The elapsed time is shown on the abscissa, and on the ordinate the relative decrease associated with submitting the leaf to a $\lambda = 515m\mu$, induced by red activated light. The leaf was exposed for three seconds at intervals of ten or twenty minutes. It can be seen from the diagram that at the end of approximately two hours of passing nitrogen through the box, the observed increase in the spectrum change was 4-5 times less than that observed in the presence of ordinary air.

It can be assumed that the passage of nitrogen through the box for two hours led to the appearance of irreversible changes in the plants, and that these changes were responsible for the observed decrease in the spectrum changes. However, as can be seen in the diagram, the effect of air which had been reintroduced into the box was already observable on the first measurement made after the reintroduction; the effect approximately equalled the effect of air at the beginning of the experiment.

Inasmuch as carbon dioxide as well as oxygen was absent in this experiment, the possibility is not excluded that the absence of carbon dioxide was responsible for the observed decrease in the zone of absorption. However, the experiments in which carbon dioxide was added to the nitrogen gave similar results. Besides this, it was determined in separate experiments that the presence or absence of carbon dioxide in normal air does not influence the size of the spectrum change caused by light. Thus, our discovery of the decrease in the size of the spectrum effect depends on the absence of oxygen, and not on the absence of carbon dioxide.

On the basis of the experiments described above, we arrived at the conclusion that the band of absorption at 515mμ, which appears on the exposure of leaves to light, decreases as the conditions change toward anaerobic.

At first glance, it appears that this result contradicts the work of Chance and Strehler [2]. It was shown in the work of these authors that the band of absorption at 515mμ that appears when an anaerobic suspension of algae genus Chlorella, is exposed to red light is bigger than that developing from the exposure of an aerobic suspension. However, this contradiction means, if it is considered, that on exposing an anaerobic (emphasizing anaerobic) suspension, a light-induced increase in absorption associated with 515mμ is stipulated for the two different processes, as noted by Chance and Strehler.

For the first process, the light caused the generation of oxygen by means of photosynthesis and this oxygen, in its turn, caused a relatively large increase in absorption for 515mμ. Thus, this spectrum change was caused by oxygen, and light is necessary for the formation of oxygen. To prove this conclusion, Chance and Strehler performed other experiments in which they showed that the spectrum change definitely may be induced in the dark (more exactly with the presence of a very weak beam of light necessary for the stated changes in spectrum absorption), if oxygen is added to the anaerobic suspension. The second process, which caused an increase in the absorption for 515mμ, was observed in aerobic conditions on the exposure of the suspension to intense light. The size of this spectrum change is less than that which is obtained on the switch from anaerobic to aerobic conditions. In this second process, light serves not only as a medium in the formation of oxygen, but also as a necessary condition for spectrum changes.

Apparently, the necessary degree of anaerobiosis was not obtained in the present work, and all observed spectrum changes were exposed immediately to a second aerobic process, and not to changes in the pressure of oxygen, because of the exposure of the leaf. The absence of a deep anaerobiosis of the type that Chance and Strehler probably had in their work is explained not only by the great difficulty of removing oxygen from an object such as the leaf, but also by the fact that periodically the leaf was subjected to light and therefore a certain amount of oxygen was formed in it.

On the basis of the above, we come to the conclusion that under conditions of incomplete anaerobia, the size of the light-induced and of absorption for 515mμ is decreased when the amount of oxygen is decreased. It is possible that the presence of oxygen is a necessary condition for the occurence of light-induced spectrum changes with 515mμ.

The results obtained, while showing the oxidizing-deoxidizing character in the reaction, do not permit a similar answer to the question of whether the observed spectrum product (515mμ) develops as a result of deoxidizing or as a result of oxidizing the appropriate precursor. In fact, on one hand the oxygen can be necessary for the oxidation of the precursor under conditions of light (photo-oxidation reaction), and, on the other hand, the action of the oxygen can be confined to oxidizing the precursor in the dark and also creates the condition for its own deoxidation by light (photo-deoxidizing reaction).

A choice between these two possibilities cannot be made on the basis of the presently available data.

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FROST RESISTANCE OF THE ROOT SYSTEM OF SOME WOODY PLANTS

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Transplating large trees in the winter will definitely become an accepted practice in landscaping as an important factor in the increase of the rate of planting, the end of seasonality in work, the maintenance of a constant work force, and the reduction in the cost of transplanting large trees. Thus, for example, the transplanting of trees in the winter without enclosing them in balls means, according to our data, a decrease in cost of 28 to 39% as compared with fall transplanting with trees enclosed in covered balls [4].

The problem of complete survival of trees transplanted in winter is of equal importance to the decrease in the cost of transplanting large trees. We conducted an investigation in this direction under the direction of the Landscaping Section of the Moscow Forest-Technical Institute. It was determined that among the several objectives of the landscaping section at Moscow would be the winter transplanting of small-leaved linden, smooth elm, sharp-leaved maple, grafted apple trees (Borovinka, Grushevka, and brown), cherry, common lilac, and other fruits which die as a result of the freezing of their root systems. Also studied would be prickly spruce, Siberian larch, and Siberian apple, in which the killing of the roots during winter transplanting has very seldom been observed. In connection with this, we established a series of experiments designed to examine the frost resistance of the root systems of eleven tree species. Most of these are widely used in Moscow tree plantings. A study of the frost resistance of root systems was carried on by the direct method under field and laboratory conditions. The temperatures of the frozen root systems of the tree species being investigated under field conditions and inside the ball of the transplanted tree were determined with the help of electrometers made by us: "thermo-spiders" and soil thermometers.

The "thermo-spider" instrument consisted of 10 nickel-copper thermocouples arranged in order. The hot junctions of a battery of thermocouples were placed on the end of a bamboo stick 4-5 mm in diameter and 60 cm long. Measuring was carried on in either bundle or fan form, i.e. in the first case, one hole with a diameter of 22 mm was bored, and in the second ten holes with diameters of 5.5 mm were bored. The registering part of the instrument consisted of a potentiometer with a type PP current. The electric current produced in a battery of thermocouples was measured by the compensation method in the range from 0-71 mv. A small change on the scale of the potentiometer - 0.05 mv - equalled 0.01°C.

The temperature in the compartment of a "Gazoapparat" refrigerator, which was converted by us, was determined with the help of twenty nickel-copper thermocouples. A GÉS-47 galvanometer served as a registering instrument. One mm on the scale of the galvonometer equalled 0.068°C.

For carrying out research in the refrigerator compartment of the Climatic Station of the Moscow Forest-Technical Institute, the temperature was determined with the aid of a platinum resistance thermometer, thermographs, and standard thermometers. The thermorelay in the refrigerator compartment was absolutely necessary for the study of the temperature regime.

The entire sets were frozen through under field conditions; only the root system separated from the trunk, or root cuttings 20-40 cm long, were frozen in the boxes of the Climatic Station of the Institute; and root cuttings 9-10 cm long were put in the boxes of the refrigerator "Gazoapparat". The age of the sets was 5 to 8 years and the age of the roots being studied was 2 to 5 years; the diameter of the roots was 0.5 to 2.0 cm.

Roots for study were taken from a depth of 20-50 cm. Not less than 25 sets or not less than 30 root cuttings were studied in every temperature range. Not less than 100 roots taken from 25 sets were used for the entire study. Good one year-old growth of the branches of the sets (20-50 cm), large buds, and well developed suckers attest to the normal growth and development of the study trees. The experiments continued for four winters (1953 to 1958). Each freezing of the sets or root cuttings lasted for 24 hours. From this it was determined that the speed of freezing and the speed of thawing did not cause additional damage to the living root tissue that was examined.

The presence of damage in the frozen roots was determined by putting them in wet sand at 20-24°C after freezing. After 2-3 weeks the frozen roots turned black and decomposed. The sound roots that had been exposed to low temperatures had a healthy appearance after 2-3 months, and in several species (black locust, Siberian apple) root sprouts developed.

Along with this universal method, the vitality of the tissue of these roots was determined periodically (one time per week) by dyeing the cells with a neutral 0.05% dye and with repeated plasmolysis and deplasmolysis in a molar solution of sucrose.

The results of the experiments to determine the frost resistance of the root systems of eleven tree species are as follows:

Tree species	Roots transfered to a negative temperature in °C	Roots frozen at a temperature of °C
1. Black locust	−5	−6−7
2. Horse chestnut	−8	−10−11
3. Large-leaved linden	−10	−13−14
4. Cultured apple (Borovinka, brown)	−12	−15
5. Sharp-leaved maple	−14	−16
6. Small-leaved linden	−16	−18
7. Balsam poplar	−16	−19−20
8. Warty birch	−17	−19
9. Siberian apple	−18	−23
10. Siberian larch	−19.5	−25
11. Common spruce	−28	−33

Frost resistance of roots was observed beginning in the middle of December and lasting to the first half of March. In the spring after the thawing of the soil, frost resistance of roots is slowly reduced and reaches a minimum during the summer period (during which roots are killed at temperature from −2.5 to −3.5°C).

Frost resistance of root systems in the middle of winter does not attain the level of the resistance of the above-ground portions of the tree because the roots, being located in the ground, cannot pass through both phases of hardening [1]. Because of this, we carried out a biochemical study of the living cells of roots and above-ground portions for the species of trees in the study, using the Genkel' and Oknina method [2]. This study showed that with the advance of the cold period of the year, saccharification of starch and storage of fat takes place in the above-ground portions of the tree. In the root system the starch grains are maintained for the entire winter period, sugar is stored in smaller quantities, and fats are stored in insignificant quantities.

A lipoid layer that shows up well in the branches of trees of frost-resistant species (common spruce, Siberian larch, warty birch, Siberian apple) is not found in the root cells of these trees, or is overlooked in view of the small individual drops by the tonoplast, nor in the plasmolemma of the cambium cells and adjacent young cells of the xylem and phloem. Still less of the lipoid was found in the root cells of small-leaved linden and balsam poplar.

Isolated protoplasm, such as that found in the cells of the above-ground portions, was not found in the cells of the root system. Protpolasm was isolated in the individual cells of the cambium and phloem only in the above-mentioned frost resistant species.

In contrast to the findings for above-ground portions, plasmodesma did not disappear during the entire winter period in the majority of the root cells, and plasmolysis was concave or spasmodic; but in those cells where isolation of protoplasm was observed, the time of the approach of false plasmolysis in a molar solution of potassium thiocyanate was 3-4 time smaller than in those cells of the above-ground organs of the same species.

Studies in the winter of 1955-1956 showed that frost resistance of root systems to the known degree depended on the amount of assimilation apparatus of the tree during the preceding growing season.

It was determined that the earlier the crown was removed, less hardening of the crown took place and less starch, sugar and fat were in its cells. The removal of the crown after the leaves were dropped (beginning of October) did not lower the frost resistance of the roots. We noticed this in the case of warty birch, balsam poplar, sharp-leaved maple, and small-leaved linden.

Our research, which carried on the work of Nikolaev [3], served to clear up the question of the reason for loss of some trees transplanted for landscaping purposes in the winter at Moscow. With heavy frost, it is possible to transplant only those species of trees which exhibit great frost resistance in their root systems (common spruce, Siberian larch, warty birch, Siberian apple). Such species as balsam poplar, small-leaved linden, sharp-leaved maple, and grafted apple, can be transplanted if there is no heavy freezing of the ball. For these species there must be extensive use of snow to cover the ball of the tree when trenching, storing, and planting; or transplanting must be done only on those days when the air temperature is higher than minus 15°C. We recommend the complete exclusion from winter transplanting for tree species with poor frost-resistant root systems: large-leaved linden, horse chestnut, and black locust. These species can be transplanted in the spring to fall period or in the winter during periods of prolonged thawing.

Even frost-resistant trees must be withheld from transplanting in the winter if the crowns have been damaged for any reason during the summer (pests, disease, mechanical damage, etc.).

If the soil at the place of excavation is dry, winter transplanted trees must be well watered immediately because the roots, while not totally damaged, need constant moisture in order to survive.

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THE INTERRELATIONSHIP BETWEEN ROOT AND FOLIAR NUTRITION AND PHOTOSYNTHESIS IN PLANTS

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The study of problems pertaining to the foliar nutrition of plants has led to the conclusion that the nutrition of plants through the leaves is very closely interrelated with the entire complex of the major physiological processes, including photosynthesis, respiration, enzyme activity and, of especial interest and importance, with the root nutrition of plants [1]. The relation of foliar nutrition to photosynthesis, respiration, enzyme activity, growth, and plant development was also established by several other authors [2-16]. As for the relationship between the nutrition of the roots and foliar nutrition of plants, until recently concern has been only with the relationship of the effectiveness of foliar nutrition (foliar feeding) on the degree to which the plants are supplied with food, and water which the plants absorb from the substrata by the roots [17-19]. Only in very recent years has the effect of foliar nutrition on the process of absorption and utilization of mineral nutrients by the root system been established by means of investigations by members of the plant physiology faculty of the Khar'kov Agricultural Institute. In Shereverya's greenhouse experiment [19] summer wheat Artemovka which received supplementary foliar nitrogen feeding during the period of stooling and heading not only accumulated more nitrogen (+54.5%) in its aerial organs, but also more phosphorus (+23.1%) and potassium (+20.6%). Shereverya [19] came to the conclusion that foliar feeding could be considered as an agricultural procedure which could be used to increases the effectiveness of fertilizers added to the soil, as well as the effective use of naturally occurring soil nutrients. The facts established by N. I. Shereverya needed verification and elucidation.

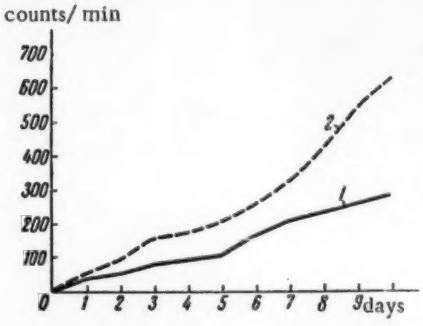

Fig. 1. The dynamics of the entrance of radioactive isotopes of phosphorus into tomato leaves (1956 experiments).
1) Control plants; 2) plants which received supplementary foliar feeding of a urea solution.

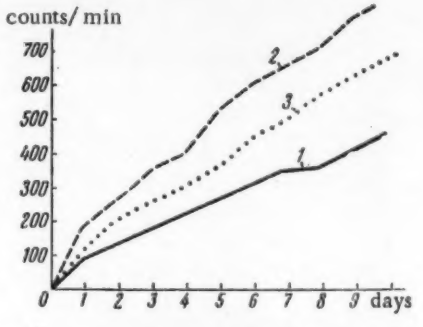

Fig. 2. The dynamics of the entrance of radioactive isotopes of phosphorus into tomato leaves (1957 experiments).
1) Control plants; 2) plants which received a supplementary foliar feeding of a urea solution; 3) plants which received a supplementary foliar feeding of a phosphate solution.

TABLE 1

Nitrogen Content, in Percentage, of the Aerial Organs and the Roots of Control Plants and Those which Received a Foliar Feeding of a Urea Solution

Experimental variants	Leaves				Stems				Roots			
	1	2	3	average	1	2	3	average	1	2	3	average
Control	3.54	3.88	3.52	3.65	1.56	1.31	1.40	1.42	2.96	2.69	2.62	2.71
Foliar feeding	4.24	4.32	4.20	4.25	3.30	3.20	3.89	3.13	3.01	3.42	3.00	3.14

TABLE 2

Nitrogen and Potassium Contents, in Percentage, of the Aerial Organs and Roots of Control Plants and Those which Received a Foliar Feeding of Urea Solution and KH_2PO_4 Solution
1957 experiments;(average of three experiments)

Variants	Leaves		Stems		Roots	
	N	K	N	K	N	K
Control	2.16	0.46	1.70	0.41	1.38	0.37
Feeding with urea solution	4.31	0.64	2.48	0.59	2.68	0.57
Feeding with KH_2PO_4 solution	3.29	0.90	1.36	0.66	2.71	0.69

TABLE 3

Rate of Respiration in Roots of Tomato Plants — Controls and Those which Received Supplementary Foliar Feeding of Urea and KH_2PO_4 (in mg CO_2)(1957 experiment)

Experimental variants	In mg CO_2/g for 1 hr	In %
Control	11.05	100
Nitrogen feeding	11.96	108.2
Phosphorus feeding	12.81	115.0

TABLE 4

The Rate of Photosynthesis, in Control Tomato Plants and Those which Received Supplementary Feeding Through the Leaves, Ten Days after Feeding (1 g/m^2 for 1 hr)(1956 experiment)

Experimental variants	No. of experiment			Average	
	1	2	3	in g	in %
Control	0.10	0.18	0.11	0.13	100
Foliar feeding	0.24	0.26	0.19	0.23	177

In 1956 and 1957 we performed a series of experiments on water cultures of tomato plants, variety Mayak. The plants were grown on Hellriegel's nutrient solution until they started to form buds, then the absorbing surface of their root system was determined using Kolosov's method [20]; only those specimens in which the absorbing surface of the roots was quite similar were chosen to be used in further experiments.

The plants chosen in 1956 were divided into two groups, control and experimental, and in 1957 we chose three groups of plants (16 plants in each group). All the plants were placed in Hellriegel's nutrient solution to which a radioactive isotope of phosphorus (P^{32}) was added as a tracer at the rate of 20μ C per vessel. Then a group of experimental plants received a foliar feeding of a 1% solution of urea; they were sprayed with an atomizer; each plant received 4 ml of urea solution. The second group of experimental plants received a foliar

feeding of phosphorus in the form of a 1% solution of KH_2PO_4; a third group was sprinkled with distilled water (control). In the last 10 days the amount of radioactive phosphorus in the leaves (in one and the same leaf layer) which had been absorbed by the roots from the nutrient solution was determined daily (the accumulating total); the determinations were made with a Geiger-Müller counter and a conversion apparatus type B (PS-64). The amount of P^{32} which was absorbed by the roots and entered the leaves was calculated on the basis of the number of counts in one minute. In the calculations obtained (average of 10) a correction was made for the natural radioactive disintegration of P^{32} during the elapsing time period. The results of experiments performed in the summer of 1956 are given in Fig. 1, and those of the 1957 experiment in Fig. 2. The data indicate that foliar feeding accelerated the absorption of phosphorus by plant roots.

An analysis of the plant material for nitrogen and potassium content* after the experiments were terminated in 1956 and 1957 revealed that they had been absorbed more readily by plants which had received foliar feeding than by the controls (Tables 1 and 2).

Hence, the facts established by N. I. Shereverya in 1955 with summer wheat were confirmed in our experiments with tomato.

In what way does foliar feeding affect absorption of mineral nutrients from the substrata by the plant roots? It is known that roots utilize food and respire at the expense of photosynthetic products. Naturally, the greater the intensity of photosynthesis, the more organic substances the roots will receive, the more intensive will be their growth, and the more rapidly will their absorbing surface increase, so that in turn there will be an increase in the absorption of mineral substances. At the same time, the more abundant flow of energy-supplying substances from the leaves to the roots should bring about an increase in the rate of respiration, which we did observe in our experiments (Table 3). The increase in respiration, in itself, can be the cause of a more intensive absorption of mineral substances from the nutient solution by the roots.

As we have already noted above, it has long been known that foliar feedings, as a rule, very substantially increase the rate of photosynthesis.

In the experiments described above it was also observed that in all three instances the rate of photosynthesis was higher in plants which received supplementary foliar feeding than it was in the controls; the average increase amounted to 77% (Table 4). Similar data were obtained in the 1957 experiments.

The rate of photosynthesis was determined using the weight method at three different times – at the end of the first, second, and third experiments in 1956, and the three experiments in 1957. In this way the presence of a close relation between foliar feeding, photosynthesis, and the absorption of mineral substances from the substrata by the roots was confirmed.

The work was done at the physiology department of the V. V. Dokuchaev Kharkov Agricultural Institute under the direction of Prof. F. F. Matskov.

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NUCLEIC ACIDS DURING THE FORMATION AND GROWTH OF SEEDS IN FRUIT CROPS

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At the present time the significant role of nucleic acids, DNA and RNA, in the most important life processes of plant and animal organisms has been established.

Their active participation in protein synthesis [1 and others], in growth and developmental processes [2-4], in the healing of wounds and grafts [5, 6], etc. has been demonstrated. This is confirmed by their concentration in areas of greatest physiological activity (embryological, glandular, and other tissues).

However, the question of nucleic acid content in plant tissues passing over into the dormant stage or else already in this condition remains inadequately answered. The literature contains data pertaining to the content of nucleic acids in buds and cambium of woody plants [7, 8a, 9], in potato tubers, in the roots of rubber producing plants, and various bulbous plants [10] in either the vegetative or dormant stage.

The existing data, with a few exceptions, indicate that there is a marked decrease in the nucleic acid content, especially RNA, of plant tissue during its transition to the dormant stage and an increase in their content during its transition from dormancy and the renewal of growth processes. Very few investigations have been concerned with the problem of nucleic acid content of seeds during the process of seed formation, growth, and transition to the dormant stage [8b, 11]. Whereas many aspects of the physiology of seed dormancy have been studied quite thoroughly [12-15 and others], the problem concerning the activity of nucleic acids in the seeds remains hardly touched.

The object of the present investigation was to study the activity of nucleic acids during the processes of formulation, growth, and transition to the dormant stage of the embryo in the seeds of two varieties of cherry, Lyubskaya and Polevka, and Chinese apple.

Pollinated ovaries, young fruits, and the embryos within the seeds of ripe fruits were studied, the material was fixed in Helly's liquid every 3-7 days, from May to August. Permanent preparations were prepared using accepted microtechnique methods. In order to determine the presence of DNA in the tissues sections were stained with basic fuchsin(Schiff's reagent) according to Feulgen, and with methyl green according to Win to disclose the presence of RNA. In order to prepare the methyl green, pyronin methyl green which had first been purified of any possible contamination by methyl violet was used [16]. The preparations were stained with a corresponding control. Before being mounted in Canada balsam the preparations were first dehydrated in an acetone-xylol series, instead of the customarily used alcohol-xylol series.

In cherry and apple soon after flowering and pollination at the end of May, fruits begin to form. At the beginning of June in the young undifferentiated embryo and strong DNA reaction was observed, as well as very strong RNA reaction. In the actively dividing meristematic tissue, the cell plasma and the large nucleoli were saturated with RNA, DNA was concentrated in the nuclei.

In the early stages of embryo formation, during all of June, when the cotyledon initials appeared, the intensity of the DNA and RNA reactions remained unchanged. In July, in the seed one could already see the large embryo which had displaced practically the entire endosperm; the cotyledons had lengthened considerably and between them the epicotyl was clearly distinguished; procambium tissue was initiated in the hypocotyl and cotyledons.

Depending on the degree of development and growth of the embryo, its nucleic acid content decreased gradually, primarily at the expense of RNA. For a time a considerable amount of DNA and RNA was retained in the procambium of the cotyledons and radicle, in the growing tip of the epicotyl, and in the dermatogen of the embryo.

At the end of July the cherry embryo was completely formed and had attained the usual dimensions found in mature seeds. At this time the fruit began to ripen. In apple such maturation of the embryo and ripening of the fruit began later.

While ripening, the tissues of the embryo gradually became dormant. At the end of July in cherry, and in August in apple, a separation of the protoplasm was noted in the cells, an accumulation of storage food substances, loss of the usual spherical form by the nuclei, and the change to a somewhat ovular contour. During the transition to the dormant stage the nucleic acid content decreased markedly, whereupon the reaction to DNA became less than the average, and the reaction to RNA in many instances was negative. In mature seeds appreciable nucleic activity was noted in the procambium, epicotyl, and dermatogen, but it was weaker than in the developing embryos.

Hence, one can show experimentally that the nucleic acid content is high during the early stages of embryo growth and development when the greatest number of various stages of mitosis is noted in the tissues. In the formed embryo, when the growth processes slow down, the content of nucleic acids drops noticeably, especially that of RNA. While the embryo cells are becoming dormant and cell division subsides the DNA content becomes low, and RNA practically disappears completely. DNA and RNA are retained in small amounts in the growing points, in the procambium, and in the dermatogen of the embryo. Some reserve of nucleic acids in the dormant cells of the indicated tissues should, apparently be considered as an energy supply for the weakening processes, which are essential for the renewal of growth during seed germination.

The indicated activity of nucleic acids in the processes of development, growth, and transition to the dormant stage in the embryonic tissues of seeds, is evidence of the important role nucleic acids play in growth and developmental processes.

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MICROSPOROGENESIS AND CAROTENOIDS

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The wide distribution of carotenoids in the plant and animal kingdoms has not yet been fully explained. There is an indication that these pigments participate in the photosynthetic process [2], that there is a relationship between them in nonplastid form and the reproductive organs of plants [3, 4], and that they are present in reproductive organs of animals [5].

The object of our investigation (carried out under the direction of P. M. Zhukovskii) was, first, to trace the dynamics of carotenoid accumulation in the anthers of a number of phylogenetically unrelated flowering plants from the bud stage to the time of flowering, and, second, to investigate the activity of carotenoids in the anthers at various stages of pollen development, starting with the formation of the pollen mother cell, i.e. from the time of early microsporogenesis.

The carotenoids were extracted using Murri's method [6] with slight variations. The pigments were determined quantitatively with the help of a concentrating colorimeter KOL-1M, and in Lilium henryi with a Shtuffen-photometer with a blue filter S-47.

The presence of carotenoids in the pollen grains was first disclosed by Bertrand and Poirault [7]. The works of Karrer [8-11], Zhukovskii and Medvedev [4-12], Lebedev [13], and other authors contain an indication that carotenoids are present in the pollen and anthers of plants. Some of the papers also contain an indication that there is an increase in the carotenoid content of the reproductive organs up to the time of flowering [12-16].

Table 1 contains the results of our analyses of the carotenoid content of 24 species of flowering plants belonging to 10 orders. From the table, one can see that in the species found at different stages of phylogenetic development there was an increase in carotenoid content from the bud stage to the time of flowering (the stages were distinguished by the degree of maturity of the formed pollen grains).

This relationship could be seen in the more primitive order Ranales, as well as in the more advanced representatives of dicotyledenous plants belonging to the Compositae family. Thus, it was shown that in several species of flowering plants located at various phylogenetic levels in an order there was a steady accumulation of carotenoids depending on the degree of maturity of the formed pollen grains in the anthers.

In two specimens, Lilium regale and L. henryi, the carotenoid content of the anthers was determined during their stages of development beginning at the time of the formation of pollen grain mother cells and ending at the stage when the pollen is changed from the tetrad to the uninuclear stage (i.e. in the microspore condition).

In both species of lily, the condition of the pollen mother cells in the anthers of each bud was checked with acetic-acid acetocarmine. It was first established that all the anthers in a flower were at about the same stage of development. From this bud the anthers were then taken for a biochemical analysis and for fixing with acetic-acid alcohol, in preparation for the later making of permanent slides from which the given microphotographs were made (see Fig. 1).

In Lilium regale the analysis was begun from the time pollen mother cells were in the early prophase

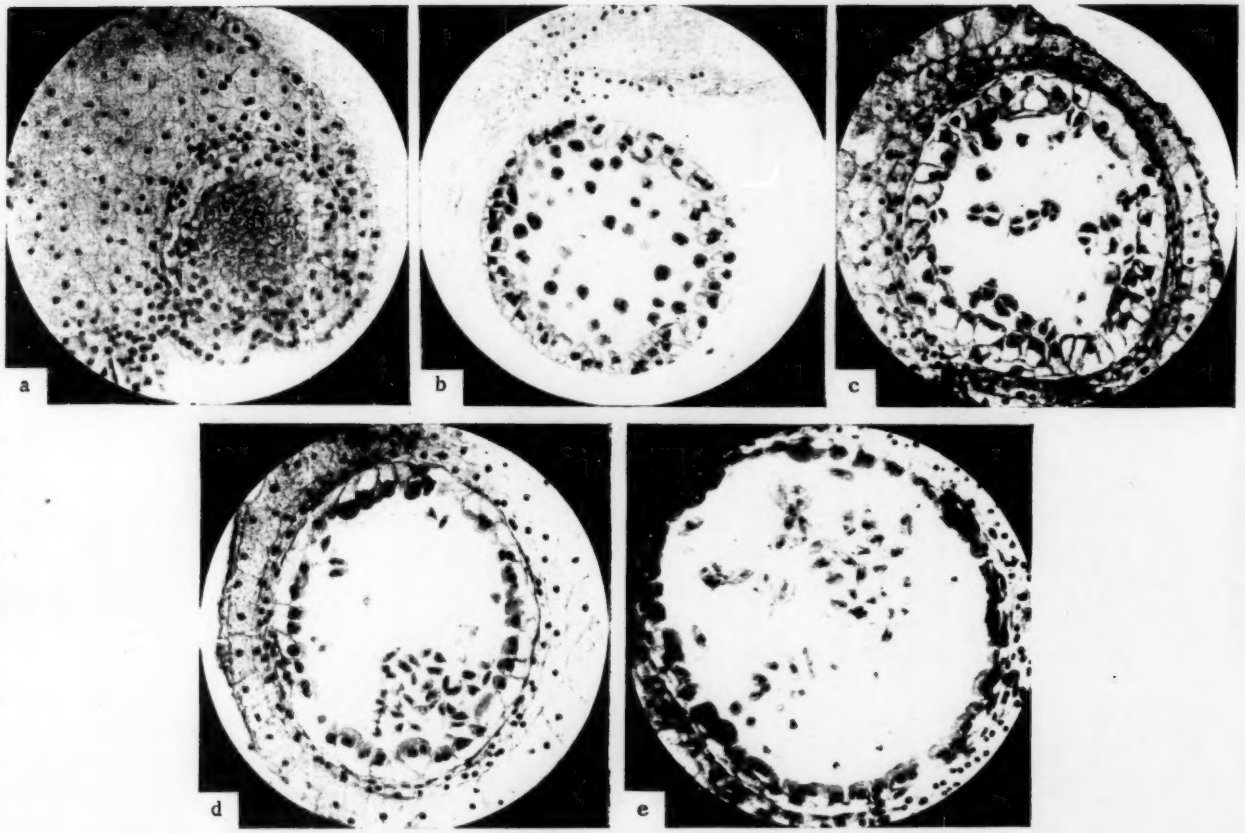

Fig. 1. Stages in the formation of pollen grains in Lilium Henryi starting with the spore mother cells. Fixed in a mixture of 70% alcohol and glacial acetic acid (10 : 0.5), stained with iron haematoxylin according to Heidenhain, enlarged 150 times: a) spore mother cells formed in the anther; b) reduction division in the spore mother cells (metaphase of the first division); c) formation of dyads and tetrads; d) young microspores, immediately following the tetrad; e) uninuclear microspores just formed from the tetrad; e) uninuclear microspores with a fully formed structural membrane.

TABLE 1

Carotenoid Content and Activity in the Anther of Some Species of Flowering Plants During the Bud Stage and During Flowering

Order	Species	Carotenoid content in mg per 100 g material			
		fresh		dry	
		buds	flowers	buds	flowers
Ranales	Laurus nobilis	10.5	13.25	56.1	67.5
	Amygdalis commun	13.2	16.0	–	–
Rosales	Persica vulgaris, Nikitskii variety Él'berta	14.7	20.2	–	–
	Cerasus syium, variety Monomakh	28.2	30.2	–	–
	Rosa canina	23.1	28.3	89	98
	Lilium regale	4.2	113		
Liliales	Lilium henryi	10.3	72.7	52.2	414
Leguminosales	Laburnum anagiroides	32.4	38.7	115.4	143.9
	Spartium junceum	5.65	6.7	20.0	21.1
Anacardiales	Poncirus trifoliata	4.3	5.7	22.9	31.3
	Citrus sinen sis, variety Gamlin	6.6	19.1	30.2	89.3
	Citrus reticulata, variety Natsumikan	3.9	11.5	23	68
	Citrus limonia, variety Novogruzinskii	5.4	9.4	34.0	61.2
	Citrus junos	10.5	13.3	56.1	67.4
	Pistacia vera	4.8	5.8	19.2	26.9
	Pistacia mutica	7.4	10.2	30.9	42.4
Rhoeadales	Papaver orientale	1.46	2.2	6.2	12.2
Tubiflorae	Solanum rybinii	5.8	12.5	41.7	73.4
	Solanum demissum	9.2	24.4	–	–
	Solanum tuberosum, variety Kameraz	2.9	8.6	22.1	58.0
	Orbanche hederae	4.3	8.0	–	–
Cistales	Cistus tauricus	64.0	82.5	170	190
Juglandales	Juglans regia	2.65	3.0	16.7	20.1
Asterales Compositae	Helianthus annuus	50.3	61.1	–	–

(synapsis), and the chromosomes were clearly visible as thin twisted threads (first phase). The characteristics of the second stage were that the pollen mother cells were in the metaphase of the first division or in the form of tetrads (the first ones predominated). The third phase was the young uninuclear pollen grain (microspore), which had just emerged from the tetrad and had not yet lost the angular form. The fourth phase was the uninuclear microspore with a fully formed structural wall.

As one can see from Table 2, the concentration of carotenoids decreased markedly in the second phase (i.e. during reduction division and the formation of tetrad). Toward the time of the formation of the uninuclear pollen grain (phase III-IV) the amount of carotenoids increased.

In Lilium henryi the carotenoid content was determined during the following phases: Phase I (Fig. 1,a)– the pollen mother cells had already developed in the anther; Phase II (Fig. 1,b) – the metaphase of the first division was occurring in the pollen mother cells; Phase III (Fig. 1,c) – during the formation of the diad and tetrad; Phase IV (Fig. 1,d) – the microspores were formed (young pollen grains), they had just emerged from the tetrad; Phase V (Fig. 1,e) – uninuclear microspores with a fully formed structural wall.

From Table 3 one can see that in Lilium henryi there was a decrease in carotenoid content toward the second phase (i.e. the metaphase of the first division). This decrease became even more noticeable toward the time of the diad and tetrad formation (phase III). The quantitative decrease of carotenoids at the time of meiosis during microsporogenesis may be explained by their disintegration and the subsequent utilization

of disintegration products as a source of energy during reduction division (Zhukovskii [3]). From the time the uninuclear pollen grain was formed there was an accumulation of carotenoids in the anthers (phases IV, V). The carotenoid content in mg per 100 g dry weight remained practically unchanged in the aforementioned phases of L. henryi (Table 3).

TABLE 2

Carotenoid Content of the Anthers During Pollen Development in Lilium regale

Type of analysis	Phases			
	I	II	III	IV
Carotenoid content in mg per 100 g fresh weight	4.8	2	4.2	8.5
The weight of one anther in mg fresh weight	23	36	60	68

TABLE 3

Carotenoid Content of the Anthers During Pollen Development in Lilium henryi

Type of analysis		Phases				
		I	II	III	IV	V
Carotenoid content in mg per 100 g weight	fresh	8.1	7.2	6.4	10.3	23.3
	dry	45	40.0	32.7	52.2	123.2
Weight of one anther in mg	fresh	3.6	25	36.4	43	75
	dry	0.65	4.5	7.1	8.5	14.2

From Tables 2 and 3 it is evident that in both species of lily the weight of anthers did not increase in a regular gradient during the different phases. One should note that Lilium regale and L. henryi differ from each other in several morphological features, in the number of chromosomes in the diploid phase, and accordingly belong to two different sections of the Lilium family [17, 18].

Therefore, the facts presented above lead us to conclude that in both species of lily, in spite of their different position systematically and their morphological differences, the rhythm of carotenoid content coincides with various stages of microsporogenesis. This is an indication that carotenoids accumulate not only during the process of pollen maturation, but also in the stage of late microsoprogenesis (Table 2). Apparently, these pigments also play some kind of an essential role during the transition from the diploid phase to the haploid phase. This transition, as we know, is associated with the reduction of chromatin. Of course, in order to reach such a conclusion decisevely it is necessary to obtain confirmation from many other and different plants.

The cautiously stated opinion of P. M. Zhukovskii concerning the energy relation of carotenoids to microsporogenesis indicates the need of further investigations.

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A STUDY OF THE CAUSES OF VARIABILITY IN THE DEVELOPMENT OF THE WHEAT GRAIN

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In spite of the vast amount of material concerning the nature of grain formation in different varieties of wheat, the causes of variability in grain formation in various parts of the spike have not yet been clarified; that is, the variation in the formation of grains on the same plant depending on their location within the spike. As the data of other investigators as well as our own show, the spikelets within one spike are not biologically the same. Whereas the spikelets in the central part of the spike form a large number of grains with a high specific weight, the spikelets at the upper and lower parts of the spike form smaller grains with a lower specific weight. Therefore, the physiological processes of grain formation in different parts of the spike must also vary. Sabinin [1] considered that a variation in the utilization of mineral nutrients within these spikelets might be the basis of the physiological variations in the spikelets at different parts of the spike. Variations in the availability of water to various parts of the spike can also be significant in an investigation concerning the physiological characteristics of grain formation.

Sokolova [2] came to the conclusion that the grains in the middle part of the spike could more readily obtain food substances, and therefore they were larger and had a greater specific weight. Tumanov [3] showed that the lower and upper spikelets produced a lower yield, and if the number of grains in the spike of such plants were reduced one could greatly improve the utilization of food substances by the remaining grains, since the food would be distributed among fewer grains. However, in the present experiments the author has been unable to bring the size of the grains in the top spikelets up to the level of the grains on the central spikelets, and has concluded that under favorable external conditions the size of the grains is determined not by the inadequate supply of food substances in the plant, but by the conditions at a much earlier period, before flowering, when the embryonic spike is being formed.

On the basis of such premises, it would be interesting to see what effect trimming the spikelets from various parts of the spike, and removing the side shoots and the leaves of the upper layers would have on the ripening of the grain. Fifty plants were used for removing the upper leaves and side shoots, and 10 spikes for removing the spikelets. The total water content of the grains from various parts of the spike was measured: the upper middle, and lower parts. It was assumed that the top 4-6 spikelets constituted the upper part of the spike, the same number constituted the lower part, and that all the remaining ones were the middle part; at first only the upper spikelets were left, then the lower ones, and finally the middle ones.

The leaves were removed as follows: the topmost leaf, three upper leaves, all leaves removed, all side shoots removed, and the control without any leaves removed. The investigation was made under field conditions in 1952 and 1953 with the following varieties of summer wheat; soft-Lyutestsens 62, Mil'turum 321 and hard- Gordeiforme 189 and 10. At the same time that samples of spikelets were taken to determine the water content of the grains, bunches of 50 spikes were also picked to determine the specific weight of the grains at various stages of maturity. The varieties of soft wheat were pruned during full flowering, and those of hard wheat at the onset of flowering. For abbreviation Table 1 contains only data for hard wheat in 1953.

In Table 1 one can see that removing the leaves during flowering had an effect on the water content of the grains at different periods of maturity. In most cases the grains in the lower part of the spike contained

TABLE 1

The Effect of Removing the Leaves and Side Shoots on the Water Content of the Grain (in %)

Variants of experiment	Part of spike	Gordeiforme 189			Gordeiforme 10			
		July 15	Aug. 1	Aug. 10	July 20	Aug. 4	Aug. 15	Aug. 24
One upper leaf removed	Upper	77.0	52.1	14.9	74.0	57.2	43.8	12.3
	Middle	77.0	54.7	19.8	74.5	56.4	44.8	18.7
	Lower	75.7	55.1	22.1	74.0	57.6	44.1	17.0
Two upper leaves removed	Upper	71.0	49.4	15.1	74.9	56.0	39.7	12.3
	Middle	73.0	50.0	14.6	77.0	55.9	41.5	16.7
	Lower	72.0	55.6	15.8	76.9	57.5	41.2	15.8
Three upper leaves removed	Upper	74.5	47.3	11.0	77.5	59.5	40.2	14.6
	Middle	73.0	49.4	12.2	74.9	50.7	41.9	11.7
	Lower	74.0	50.7	14.4	75.1	62.8	43.1	12.6
All leaves removed	Upper	71.5	46.5	9.6	–	–	44.2	16.5
	Middle	73.0	46.8	10.4	–	–	44.9	21.5
	Lower	73.4	47.6	11.1	–	–	48.1	20.4
All side shoots removed	Upper	73.0	–	18.3	–	–	–	–
	Middle	73.0	–	26.3	–	–	–	–
	Lower	74.8	–	30.7	–	–	–	–
Control (no parts removed)	Upper	72.8	46.8	24.7	74.5	57.3	39.1	19.7
	Middle	72.8	48.6	33.6	73.8	53.1	46.4	27.8
	Lower	74.5	50.1	30.3	74.0	60.0	–	30.4

TABLE 2

The Effect of Removing the Spikelets on the Water Content (in %) of the Grains During Various Stages of Maturity

Remaining spikelets	Gordeiforme 189			Gordeiforme 10		
	July 15	Aug. 1	Aug. 10	July 20	Aug. 4	Aug. 16
Upper	73.6	58.6	11.4	75.7	57.7	32.8
Lower	74.6	47.0	7.7	75.4	57.0	25.6
Middle	73.4	41.6	20.4	72.5	52.7	41.0
Control with pruning	72.8	48.6	23.6	73.6	58.1	46.4

TABLE 3

The Effect of Removing the Spikelets from Different Parts of the Spike at Different Stages of Maturity on the Maturation of the Grains

Remaining spikelets	Gordeiforme 189			Gordeiforme 10		
	July 15	Aug. 1	Aug. 15	July 20	Aug. 4	Aug. 24
Upper	5.8	34.1	38.7	3.5	33.2	42.1
Lower	6.2	–	40.3	2.7	28.0	45.8
Middle	8.9	38.3	43.8	6.3	36.2	53.5
Control (without removal)	7.0	34.5	42.7	–	–	54.4

more water than those in the middle and upper part. We noted this feature of water distribution in the grains after the removal of the leaves both in variety Gordeiforme 189, as well as in Gordeiforme 10.

By removing the leaves and side shoots we were able to hasten the ripening process, which is associated with the more rapid decrease of water in the grain during ripening, but we were unable to change the established relationship between the different parts of the spike. Table 2 contains the results of water content determinations after the spikelets were removed.

As a rule the regularity in the characteristics of water distribution in different parts of the spike remained the same as it had during removal of the leaves.

Such a distribution of water in the grain from different parts of the spike indicates that the spikelets varied in respect to the availability of water, and that spikelets in the upper part of the spike suffered more from an inadequate supply of water than those in the middle part, and especially those in the lower part of the spike. According to our data removing the spikelets did not change the nature of the water movement between the different parts of the spike. In the upper part of the spike the grains had a lower supply of water, even though the lower spikelets, which divert the water from the upper ones, had been removed.

It was of interest to determine the relationship between removing the spikelets from different parts of the spike and the specific weight of the grains. Table 3 contains the results of these experiments.

As the data in Table 3 show removing the spikelets from different parts of the spike did not result in an increase of the specific weight of the grains in the upper and lower parts of the spike; the grains which weighed the most were in the middle part of the spike

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AFTER-EFFECTS OF HIGH AND LOW TEMPERATURES ON PHOTOSYNTHESIS IN COTTON PLANTS

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It has been shown in several investigations [1-7] that the nature of the changes in photosynthesis after the influence of temperature depends on the physiological condition of the plant, length of the exposure, limits of temperature in which the exposure occurred, etc. However, up to the present time these relationships just mentioned have not been established for any particular plant, neither have the exact conditions at which the after-effects of temperature occur, nor what the after-effects are like at one or another stage of plant development been determined.

On the basis of its origin cotton is a thermophillic crop, and temperature has a great effect on its growth, development, and productivity [8-10]. From experience it is known that after a period of low temperatures during the spring the growth of cotton is not reduced at once, but in the summer after especially hot days there is a decrease in the number of buds and bolls. These facts are apparently related to the appearance of temperature after-effects which bring about a great change in the assimilating ability of plants.

In addition to investigations which have established the basic principles of variations in photosynthesis in cotton [11-15], one might consider the problem of an experimental study of the after-effects of temperature on photosynthesis of cotton leaves, and a study of variations in photosynthesis during the growing period in relation to the stages of growth for the plant.

The present work was carried in 1954 near Tashkent on the territory Akademgorodok. Cotton plants, variety 108-F, raised in the greenhouse and under field conditions were used in the experiments.

The investigations were made during the budding, flowering, and open boll stages. The calendar dates for the beginning of the growth stages of cotton in 1954 were as follows:

Date of sowing	Budding stage	Flower stage	Open boll stage	Experiment
April 27	June 3	July 20	Aug. 21	Greenhouse
July 13	July 23	Aug. 31	Sept. 20	Field

The experiments concerned with the after-effects of temperature on photosynthesis in cotton leaves were performed as follows.

The leaves were cut off at different times of the day and at different weather conditions. Part of the excised leaves were exposed to a specific temperature in an ultrathermostat for 15 minutes. The control leaves were kept at room temperature (27-28°) for the same length of time. Naturally, under such an arrangement the rate of photosynthesis in the control leaves fluctuated from experiment to experiment.

In order that the seasonal fluctuation of photosynthesis should not be superimposed on the fluctuations due to the temperatures of the ultrathermostat, we took the value obtained for the rate of photosynthesis in the control leaves as 100% and the value obtained in the heated leaves was calculated in percentage of the control.

The duration of the exposure of the leaves in the ultrathermostat had been established with preliminary experiments in which it became clear that, during an exposure of 15 minutes, the excised leaves and the intact ones had about the same rate of gas exchange.

After the temperature treatment, sections were cut from the leaves and placed in manometric flasks for determining the rate of photosynthesis in the Warburg apparatus; this was done at a constant bath temperature of 30° and a constant illumination with six 500-watt lamps. Normally developed leaves of the middle layer along the main stem were picked for the experiments. The period when photosynthesis was being measured lasted for 10 minutes. The experimental flasks contained a carbonate-bicarbonate buffer No. 9 [16]. The after-effects of temperature on photosynthesis were studied within temperature ranges from −5° to +12° and from +30° to +51°.

The data obtained from cotton grown under field conditions indicate that the photosynthetic activity of the leaves increased after the influence of temperatures, within the limits of 29-43°. For example, after an exposure to a temperature of 40° the photosynthetic activity of the leaves increased by 60% (from 6.5 to 10.4 mg $CO_2/dm^2/hr$); after 34° it increased by 56% (from 8.0 to 12.5 mg CO_2). An exposure to a temperature of 44° brought about a decrease in the photosynthetic activity by 17% (from 8.8 to 7.3 mg CO_2/dm^2. The photosynthetic activity of the leaves decreased even more after an exposure to a temperature of 50° (from 8.4 to 1.8 mg CO_2/dm^2). A further increase in temperature up to 51° resulted in the cessation of photosynthesis. Low positive temperatures resulted in a significant decrease of photosynthetic activity in the cotton leaves. Similar results were obtained with cotton grown in the greenhouse.

On the basis of the data obtained one might conditionally divide the temperature scale into several zones; within the limits of these zones photosynthesis in cotton leaves has specific characteristics. In the region of high temperatures one can recognize the following zones: 1) The zone in which photosynthesis was stimulated within temperatures of 30° to 40°. During the budding stage the temperatures of this zone, for example 40°, brought about an increase in photosynthetic activity of the leaves of cotton grown under field conditions from 13.6 to 15.6 mg CO_2 (by 13%); during the open boll stage (greenhouse experiment) the increase was from 4.7 to 6.2 mg CO_2 (by 32%). 2) The zone in which photosynthesis was retarded − between 40° and 50°. For example, after an exposure to a temperature of 45° the photosynthetic activity of the leaves during the budding stage decreased from 10.9 to 7.6 mg CO_2 (by 30%); during the flowering stage it decreased from 13.6 to 6.5 mg CO_2 (by 44%). 3) The zone when photosynthesis stopped. The lowest rate of photosynthesis was observed at a temperature of 50°. At 51°, under the conditions of our experiments, photosynthesis ceased completely. At the same time the leaf became flexible. However respiration did not stop at this temperature.

In the region of low temperatures one can recognize the following two zones of photosynthetic activity: 1) The zone in which photosynthesis was retarded within temperature limits of +10° to −4°. For example, an exposure to a temperature of −2° resulted in a decrease of photosynthesis from 9.9 to 4.7 mg CO_2 (doubled), a temperature of −3° resulted in a decrease from 12.7 to 5.2 mg CO_2 (two and one-half times), and a temperature of 6° resulted in a decrease from 11.5 to 9.9 mg CO_2 (by 14%). 2) The zone in which the photosynthetic apparatus of the leaf was injured; we found the critical temperature to be −5°. At a lower temperature the photosynthetic activity of the leaves stopped.

We noticed individual instances of high photosynthesis at +12°. Gashkova [2] noted a stimulation of photosynthesis and respiration in mandarin oranges after short exposures (17 minutes) to a temperature of +7°. Several investigators observed a stimulating effect of low temperature on the respiration of plants after longer exposures. However the data we obtained are not adequate to set off a zone of low positive temperatures which stimulate photosynthesis.

The temperature limits which induced either a stimulating or retarding effect on photosynthesis are rather conditional. If the length of exposure to the temperature is increased, apparently the reaction of the leaf will change and the margins of the individual zones will shift. Such an assumption can be made on the basis of data obtained by Gashkova [2], who determined the rate of photosynthesis and respiration in lemon and mandarin at three different exposures (17 min, 1 hr, and 24 hr) to a temperature of 7°, and disclosed a variation in the value and direction of the gas exchange. The problem concerning the relationship of the gas exchange mechanism to the after-effects of long exposure to a specific temperature are of great interest and should be worked out experimentally. The data we obtained indicate that the nature of the reaction of the photosynthetic process

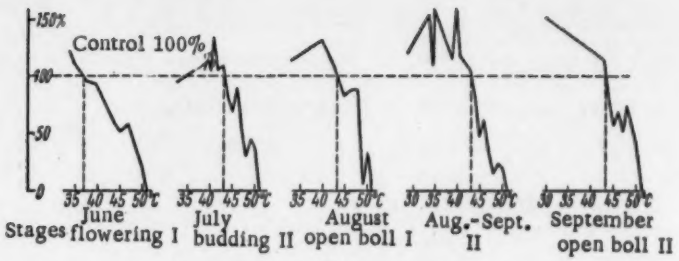

Fig. Changes in photosynthetic activity of cotton leaves subjected to high temperatures at different stages of plant development. I) Greenhouse; II field conditions.

in cotton due to the after-effects of temperature changes depended on the degree of the experimental temperature, whereupon different temperatures bring about not only various changes in the magnitude of photosynthesis, but also a variation in the direction of the changes.

The limits of the temperature zones for photosynthesis in cotton at various stages of development – budding, flowering, and open boll – can be placed along a temperature scale (see Fig.).

The top limit of the temperature zone in which photosynthesis was stimulated in plants grown in the greenhouse was 35° during the flowering stage, but it increased up to 42-43° during the open boll stage.

The shift of temperature zones in which stimulation occurred in the greenhouse experiments can be explained by comparing the given experiments in respect to the different planting dates and weather conditions, especially temperature, at which the plants grew. Until the onset of flowering the plants in the greenhouse were under normal weather conditions (maximum May – June temperature 30-32°). The stimulating zone at this stage was quite narrow and did not exceed the limit of 35°. The open boll stage was preceded by a longer period of high temperatures of 35-37°. The plants had become adjusted to the high temperature and the zone in which photosynthesis was stimulated shifted to a higher value – to 42°.

SUMMARY

According to the nature of the after-effects of high and low temperatures on the photosynthetic activity of cotton leaves three temperature zones can be distinguished: stimulation, retardation, and cessation.

The limits of the temperature zones for photosynthesis in cotton at various stages of development – budding, flowering and open boll – are distributed along the temperature scale. The experimental data indicate that the disclosed variations in the photosynthetic reaction at one and the same temperature at different stages of plant development depend primarily on the temperature conditions at which the development had occurred previously.

In conclusion, we wish to express our appreciation to a candidate in the biological sciences, O. A. Semikhatova, for all the assistance in making this investigation.

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THE INTRODUCTION OF COMPLETED WORK INTO THE NATIONAL ECONOMY

FOLIAR MICROELEMENT NUTRITION AS A MEANS FOR INCREASING YIELDS AND IMPROVING THE THE QUALITY OF STRAWBERRY FRUIT

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The effect of microelements on fruit and berry plants has been but little studied in our country. Work in this direction is unilateral and concerned almost only with graphes. Numerous investigations conducted with these plants [1-4] have shown that microelements are able to appreciably increase yield and quality of the fruits. The effect of microelements on yields, for example of the strawberry, which is one of the leading and profitable garden crops, has been dealt with by only the work of Vlasyuk, Byk and Fedchenko [5], who obtained a marked increase of yield, sugar, and vitamin C content of the fruits from the effect of manganese. In general, there has been no work on the effect of boron, zinc, copper, and molybdenum on the yield and quality of strawberry fruits.

Considering the slight study that has given to the question of the influence of microelements on the strawberry, we conducted special investigations in this direction during 1955 and 1956. The experiments were conducted on the Sovkhoz "Red Farmer" experimental station of the All-Union Institute of Plant Production with pure varieties and uniform material (variety "Novinka"). The experiments were replicated three times and had a semiproduction character, since they were carried out on large plots 70 meters long. There were 250 plants in each replicate.

The plants were fed microelements twice by foliar application: once during the budding stage, and once during flowering. The concentrations of the microelement salt solutions are shown in Table 1. Control plants were sprayed with water.

Average samples from a large number of fruits were taken for determination of acidity and carbohydrate, and vitamin C content. The content of monosaccharides, sucrose, maltose, starch, and hemicellulose was determined by the method of Bertran with the modifications of Il'ina for microanalysis. A separate determination was made of glucose (after Willstaeter) and fructose (after Kolchoff). Vitamin C content was determined by the method of Prokoshev and acidity by the generally used method of titration.

Fruit was collected separately by experimental replicate. Yield differences between replicates were 0.4-0.9 kg in 1955 and 0.1-0.5 kg in 1956 which indicates the great reliability of the data obtained.

Data on the strawberry fruit yields in 1955 and 1956 are presented in Table 2.

The data of Table 2 show that the yield of strawberry fruits is markedly increased by all microelements and combinations of them with the exception of molybdenum. Zinc, manganese, and especially the combination of boron and zinc, exert a strong influence on productivity. These microelements augmented fruit yields 21-32%. In 1955 the yield increase from the microelements occurred principally because of an increase in fruit size as indicated by analytical data on the weight of such fruits during the first picking.

An acceleration in development was observed under the influence of manganese in which 7.18 kg of fruit was collected in the first picking, while only 4.25 kg was gathered in the control.

TABLE 1

Experimental Plan and Concentrations of Microelement Salt Solutions (%)

Date	H_3BO_3	$ZnSO_4$	$KMnO_4$	$CuSO_4$	$(NH_4)_2MoO_4$	H_3BO_3 + $ZnSO_4$	H_3BO_3 + $(NH_4)_2MoO_4$
1955	0.03	0.03	0.06	0.02	0.06	0.02 + 0.02	0.02 + 0.03
1956	0.03	0.05	0.1	0.03	0.1	0.02 + 0.03	0.02 + 0.03

TABLE 2

Effect of Microelements on Strawberry Fruit Yield
(average yield per plot in kg and %)

Treatment	1955			1956	
	in kg	in %	wt. of one fruit (average of 30) g	in kg	in %
Control	41.9	100	21.4	6.1	100
H_3BO_3	45.1	107.6	21.8	10.7	176.0
$ZnSO_4$	50.8	121.3	23.4	13.3	219.1
$KMnO_4$	54.4	129.7	24.7	16.0	267.0
$CuSO_4$	48.6	113.7	23.1	11.6	191.8
$(NH_4)_2MoO_4$	42.6	101.7	21.7	7.3	120.3
H_3BO_3 + $ZnSO_4$	55.5	132.4	25.5	17.4	287.9
H_3BO_3 + $(NH_4)_2MoO_4$	45.6	108.8	22.6	12.7	207.7

TABLE 3

Meterological Conditions During July, August, and September 1954 and 1955
(average daily)

Year and Month		Air temperature, °C	Minimum soil surface temperature	Precipitation, mm	Air relative humidity
1954	July	18.0	10.8	2.6	–
	August	16.0	9.2	2.6	–
	September	11.0	4.5	1.7	60.0
1955	July	18.8	–	0.3	62.1
	August	20.4	–	0.1	55.7
	September	13.4	8.0	1.4	69.3

It is apparent from the data of Table 2 that a still more effective result was obtained with respect to increasing the yield of strawberry fruits from the effect of the microelements in 1956, when the plants experienced drying weather during July and August of the preceeding year. As we know, the next year's strawberry crop is laid down during the second half of the summer after the harvest. During this time after a brief rest there commences the growth of new leaves, the formation of additional roots, and the laying down of floral buds. Since the strawberry, the root system of which lies in the top layer of the soil, is very sensitive to a soil moisture deficiency, the drought which occurred during the second half of the summer of 1955 (Table 3) negatively affected the process of floral bud formation, as a result of which the strawberry fruit yield was severely reduced in 1956.

TABLE 4

Effect of Microelements on Strawberry Fruit Quality

Treatment	Carbohydrate content, % wet weight of fruit								Vitamin C, mg % of wet weight	Acidity, % citric acid in wet weight
	glucose	fructose	total monoses	sucrose	maltose fraction	starch	hemicellulose	total carbohydrates		
Experiment 1955										
Control	1.5	2.9	4.4	0.16	0	0.07	0.28	4.91	84	1.07
H_3BO_3	1.7	3.1	4.8	0.11	0	0.18	0.23	5.32	97	0.74
$ZnSO_4$	1.0	4.3	5.3	0.10	0	0.22	0.24	5.86	103	0.87
$KMnO_4$	1.4	4.4	5.8	0.07	0	0.14	0.18	6.19	99	0.92
$CuSO_4$	1.7	2.9	4.6	0.09	0	0.27	0.21	5.17	90	1.03
$(NH_4)_2MoO_4$	—	—	—	—	—	—	—	—	94	0.99
$H_3BO_3+ZnSO_4$	1.6	3.8	5.4	0.11	0	0.14	0.17	5.82	115	0.80
$H_3BO_3+(NH_4)_2MoO_4$	2.3	1.7	4.0	0.14	0	0.06	0.24	4.44	86	0.80
Experiment 1956										
Control	2.6	3.1	5.7	0.21	0	0.12	0.22	6.25	92	1.19
H_3BO_3	2.3	3.5	5.8	0.15	0	0.14	0.11	6.20	93	1.11
$ZnSO_4$	2.1	4.8	6.9	0.17	0	0.19	0.11	7.37	119	1.00
$KMnO_4$	2.9	3.7	6.6	0.12	0	0.16	0.11	6.99	118	0.99
$CuSO_4$	2.9	3.5	6.4	0.12	0	0.14	0.20	6.86	99	1.11
$(NH_4)_2MoO_4$	3.2	3.0	6.2	0.17	0	0.12	0.19	6.68	96	0.99
$H_3BO_3+ZnSO_4$	2.7	4.0	6.7	0.10	0	0.14	0.11	7.05	113	0.96
$H_3BO_3+(NH_4)_2MoO_4$	3.6	2.5	6.1	0.15	0	0.17	0.20	6.62	87	1.14

Six to seven times more fruit was harvested in 1955 than in 1956, as can be seen from Table 2. The decrease in yield occurred because of a decrease in their size, which in itself entailed an appreciable reduction in the duration of the ripening stage (in 1955 it was 37 days, and in 1956 only 22 days).

The negative effect of drought at the time of floral bud formation was appreciably decreased by the microelements. During 1956 all of the microelements boosted fruit yields several times above those of 1955. In 1956, as also before, zinc, manganese, and the boron-zinc combination gave the best results; yields were boosted 2-3 times under their influence.

The data we have obtained on the effect of boron, zinc, manganese and copper, in decreasing the negative effect of drought agrees with literature data obtained on other plants, involving the increase of drought tolerance under the influence of the microelements indicated [6-12].

By intensifying the efflux of sugars from the leaves and increasing the synthesis activity of the green fruits both in 1955 and 1956, the microelements increased the carbohydrate contents of the green fruits (Table 4). The carbohydrate content increase was predominantly fructose.

Under the influence of all the microelements except zinc the glucose content changed little. Zinc decreased the glucose content of the fruits both in 1955 and 1956; the boron-molybdenum combination, on the other hand, increased the content of glucose.

The appreciable increase in fructose content under the influence of zinc, manganese and the boron-zinc combination has an important value for rating fruit taste quality, since fructose is one of the sweet sugars. Such a taste rating of sugars according to Richter (cited by Kokin [13]): glucose : sucrose : fructose = 100 : 145 : 220.

It is interesting that molybdenum and the boron-molybdenum combination, in contrast to zinc, manganese, and the boron-zinc combination shifted the ratio glucose : fructose to the glucose side.

It is apparent from the data shown in Table 4 that the microelements decreased the amount of sucrose and hemicellulose in the fruits and increased the starch content.

It is essential to note that in 1956, when the yield was appreciably lower than in 1955, the strawberry fruits typically had a greater sugar content in all treatments.

It is known what a large role is played by vitamin C and the organic acids in the taste quality rating of fruits. In this connection it is interesting to note that, as is evident from the material shown in Table 4, the microelements increased the vitamin C content of the fruits and decreased their acidity. The strongest increase in vitamin C content and decrease in acidity was observed under the influence of zinc, manganese, and the boron-zinc combination.

Our data on the increase of sugar and decrease of strawberry fruit acidity under the influence of zinc agrees with the data of Dobrolyubskii [4] obtained on grapes.

The great reliability we obtained for two years with foliar application of zinc, manganese, and the boron-zinc combination to strawberries, the additional yield of fruit of larger size, especially under conditions where the floral buds are laid down under drought conditions, and also the good concurrence during the given years of the influence of indicated microelements on the quality of the strawberry fruits, permits us to recommend this technique for wide testing in production.

We consider it to be our obligation to thank the scientific staff of the experiment station of the All-Union Institute of Crop Production, to candidate of biological sciences F. I. Pikhoto, and agronomist K. A. Kiselov for great assistance in conducting our experiments.

SUMMARY

Foliar nutrition of strawberry plants with zinc, manganese and a combination of boron and zinc is a highly effective method of enhancing the yield and improving the quality of strawberry fruits. This procedure is especially effective in those cases when the flower buds set under unfavorable, drought conditions.

Extensive practial application of the method can be recommended.

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METHODS

QUANTITATIVE DETERMINATION OF DI- AND TRICARBOXYLIC ACIDS BY PAPER CHROMATOGRAPHY

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During recent years there has been an increasing interest by physiologists and biochemists in the organic acids of plants and animals. This interest is well founded. Investigations have shown that the organic acids participate in major biological processes,such as respiration and the synthesis of protein. Organic acids take part in photosynthesis as chemical links in its early stages. A deep study of the diverse manifestations of metabolism indicates that the organic acids play a role in the syntheses and transformations of many plant materials, among them fats, about 90% of which are made up of acids.

In study of the acid metabolism a marked similarity appears both in the lower and the higher plants. However, only the acid constitution of their fruits and berries has been substantially studied; the vegetative organs have been investigated exceedingly little with respect to acids. Recent quantitative investigations show that cultivated plants contain,in their vegetative parts,a very great diversity in the salts of organic acids. Here they occur in appreciable quantities, especially in legumes [1, 2]. Two acids, malic and citric, have a very wide distribution in plants; they invariably accompany one another; in addition to them, the leaves of grasses contain a predominate amount of aconitic acid [3] which is absent in beans; in the latter malonic acid [4] is present in appreciable amounts; in the onion succinic is present in addition to malic and citric acids. Acetic acid has also been found in all of the higher plants that have been investigated.

As recognized long ago, we have also discovered in the leaves of legumes new acids which are very close sugar derivatives, products of the initial oxidation or oxidation-reduction isomers of sugars [5]. Polyoxy acids have been isolated as first products of photosynthesis [6].

The acids already studied in the higher plants are appreciably more diverse than the collection of acids included in the so-called Krebs cycle. It is difficult to assume that acids not involved in this cycle do not participate in the respiration process; if only because the presence of malonic acid in legumes causes one to doubt that the process of respiration in legumes is completed only through the cycle of di- and tricarboxylic acids. The presence of acids derived from sugars provides a basis for supposing that the process of direct oxidation of sugars, by-passing the glycolytic decomposition, occurs during respiration. The possibility that there is another pathway for the formation of di- and tricarboxylic acids, other than the Krebs cycle, is pointed out by Kursanov and Kulaeva [7] in their recently published work.

Organic acids, which, after carbohydrates and proteins, predominate in their occurence in plants, are receiving the serious attention of physiologists and biochemists. Study of these acids will doubtless permit a deeper insight into the intimate, little investigated, multifaceted phenomena of metabolism in higher plants both under normal conditions,and under the influence of various kinds of effects.

It stands to reason that success in such experiments depends upon the existence of sufficiently satisfactory and practical methods for separation of the acids from the plant and determination of their chemical nature and amounts. Up to the present time, plant physiologists and plant biochemists have inclined to methods for chemically determining quantitatively small samples of only malic, citric, tartaric, and oxalic acids. For other acids taking part in the physiological-chemical processes, for example isocitric, aconitic, succinic, fumaric, and malonic, there are no dependable chemical methods of they have not been developed.

In biochemical practice during the last 15 years, completely new, original and exceedingly effective chromatographic methods have been established. Of these, thanks to its simplicity and aviliability, the method of paper chromatography, which permits the study of a great diversity of substances in microscopic quantities, has most quickly become part of laboratory practice. Paper chromatography has also found a wide use for the study of the transformations of organic acids in biological subjects. However, this method is used predominantly for separation of mixtures of acids and for a preliminary determination of their nature, but not for quantitative study. Some of the quantitative methods which have been described [8] are involved and are not without fault regarding their exactness. The method of paper chromatography permits the partition of a mixture of individual acids in the form in which they are usually extracted from the plants. However, the absolute amounts of individual acids in this separation are so low that they cannot be determined by the ordinary quantitative chemical methods.

The work presented here represents an endeavor to use the method of paper chromatography for a quantitative determination of di- and tricarboxylic plant acids without losing its very fundamental merits of availability and rare simplicity.

In the paper chromatography of organic acids most frequently a mixture of butyl alcohol, water and formic acid (18 : 9 : 2) is used as a solvent for partitioning a mixture of the acids. We have used this solvent with success for the quantitative determination of a number of acids – malonic, succinic, aconitic and fumaric. These acids are very easily separated from malic and citric. However, a mixture of malic and citric acids could not be quantitatively separated with butyl alcohol, but nearly all higher plants studied so far contain these two acids. The interconversion of these acids in plants under various conditions was the object of a series of investigations.

Malic and citric acids are partitioned comparatively easily by qualitatively chromatographing them with butyric alcohol; taken in amounts practical for titration, they partitioned on the paper in one zone without a distinct boundary between them. In order to partition a mixture of malic and citric acids in quantities practical for titration with an alkali, we used as a solvent ethyl ether, which has sometimes been used for qualitative chromotography.

The experiments led us to the conclusion that this solvent is suitable for the quantitative determination of malic, citric, and tartaric acids, essential acids in plants, not to mention succinic, aconitic, malonic and certain others.

EXPERIMENTAL METHODS

Leaves or roots for extraction of organic acids were dried at 60-70° and pulverized in a mortar or mill. A sample of the material was acidified with 80 ml of 27% sulfuric acid per 100 grams, and after thorough mixing was extracted with ethyl ether in a Soxhlet apparatus for 70-100 hours (depending on the amount of material). Water was added to the extract, the ether was boiled off, the water solution of the acids was filtered, the filter was washed, and the volume of the extract was measured. The total acidity of the solution was determined by titrating a small aliquot with 0.1 N alkali. A solution of $BaCl_2$ was added to 1 ml of the neutralized solution to react with the sulfuric acid which still, although in very few cases, can get into the extract from the holder. To the remainder of the same neutralized solution was added 0.5-1.0 ml of a strong solution of calcium acetate in order to detect oxalic acid. After precipitation occurred acetic acid was added in which calcium oxaloacetate is not soluble. An aliquot of solution was heated and small portions of a clear, saturated solution of $Ca(OH)_2$ (concentration about 0.15%) were added to it from a burette until the precipitate of calcium oxalate ceased forming.

The solution with the precipitate was heated, filtered through a fine-grained glass filter, and the residue washed. Oxalic acid was determined volumetrically by titration with permanganate. The filtrate containing the free acids was chromatographed.

Commonly, the qualitative collection of acids in plants is not large, 5-6 acids, and the quantitative ratios between them in relation to the stage of development changes strongly up to the disappearance of some acids; therefore, an orientational, qualitative chromatogram must be made (see below) using the solution of acids directly.

If the chromatogram detects the presence of 5-6 acids in sufficient quantities, as evidenced by the color intensity of the spots, the di- and tricarboxylic acids ought to be separated from the others, possibly the lactic

or derivative sugars. In order to do this, all of the acids (except oxalic) are converted into barium salts with the aqueous solution neutralized to the phenolphthalein end-point with 0.1 N $Ba(OH)_2$. The sulfuric acid is also removed, if it accidentally got into the extract. Then the solution is concentrated in a vacuum or on a water bath to 37 ml if the $Ba(OH)_2$ is less than 100 ml, or to 74 ml if the 0.1 N $Ba(OH)_2$ used in neutralization exceeds 100 ml. The concentrated solution and the precipitate formed are quantitatively transferred to a measuring vessel or graduated cylinder, brought up to 100 ml or 200 ml volume respectively with 96% alcohol and shaken up. Up to 95% of the di- and tricarboxylic acids is precipitated in the 60% alcohol solution formed. On the following day the precipitate is filtered through a small porcelain funnel (Nutsch filter), washed three times with 20 ml of 60% alcohol while it is being ground in a mortar, dried at 60-70°, weighed and stored in a desicator. Since 30-50 mg is sufficient for a quantitative chromatographic determination of di- and tricarboxylic acids, it is only necessary to consider the needs for a quantitative chromatogram and replicate analyses.

A weighed portion or all of the salt (the barium in it varies from 45-55%) is dissolved in water, heated, and the barium precipitated with a small amount of 5% sulfuric acid. The barium sulfate is filtered out and washed. The excess of sulfuric acid is removed from the filtrate with a solution of 0.1 N $Ba(OH)_2$. The acid-free filtrate is reduced by evaporation or diluted to 4-6% depending on the malic acid present. This solution is used for the qualitative (orientative) and quantitative chromatographs. For subsequent determinations of di- and tricarboxylic acids in plants, an aliquot of the solution is titrated with 0.1 N alkali, and recalculated on a volume-weight basis.

Required for the analysis:

1. Solvents. A mixture of 18 parts of medical ethyl ether, 9 parts distilled water and 5 parts formic acid by volume (18:9:5) is used as a solvent. A measured quantity of the constituents is poured into a large glass-stoppered flask and shaken about half an hour; the mixture is transferred into a separatory funnel, and after settling and stratification, the liquid layers are poured-off. The top layers containing ether saturated with water and formic acid are used.

2. Chromatographic chamber. A glass vessel with a diameter not less than 16 cm and a height of 60 cm, equipped with a glass or ground-in plastic lid can serve as a chamber. Two of the glass vessels indicated above can be used, and placed one upon the other with a total height of 60 cm (Fig. 1). In the lower vessel, to within 3-4 cm of the top edge, an opening may be drilled for a small rubber stopper in which is placed a thick-walled glass tube with a outside diameter of 4-5 mm. This tube is bent twice at right angles. The short arm is inserted into the stopper from the inside of the vessel wall, and a Bunsen plug slipped onto the outside end of this arm. The inside, vertical, long portion of the tube goes up at a distance of 2-3 cm from the vessel wall, and 2-3 cm below the top of the vessel turns at a right angle; the horizontal part of it, 12-14 cm long, serves as an arm for supporting the chromatogram. The Bunsen plug is prepared as follows: a rubber tube 6-8 cm long is slipped onto a glass tube 6-8 cm in diameter, and a longitudinal slit about 0.5 cm long is made in it with a razor. In the other end of the rubber tube is placed a short segment of glass rod, or tube sealed at one end. The plug is necessary to adjust in the chamber for excessive vapor pressure of ether, which has the rather low boiling point of 34.6°.

3. Developer – 0.05% aqueous solution of bromphenol blue.

4. Indicator – 1.0% tincture of phenolphthalein.

5. Chromatographic paper No. 4 ("slow") Volodarskii Factory, Leningrad.

Before commencing the partitioning and quantitative determination of the acids, it is necessary to ascertain which ones are found in the solution studied; this is accomplished with a qualitative chromatograph, using as references the acids more widely distributed in plants, malic, citric, aconitic, tartaric, etc. Two-tenths molar solutions of the reference acids and a strip of paper about the width of the chamber 2-3 cm narrower), for our chamber 13 cm, i.e., $^1/_4$ of a standard sheet, are prepared. A line is drawn with a pencil 2.5 cm from the narrow end of the sheet; on its points are made 1.5 cm from the side of the sheet and 3 cm from one another (5 points in all). A droplet of one of the reference acid solutions is placed on each point; the droplet loci are dried out with a fan or room ventilator. After the paper is dried, a drop of the same solution is again placed on the same point and again they are dried out. Five droplets are put on in this way; after the reference acids are put on, the solutions of acids being studied are placed on the unused points. Their concentration is reduced to 0.2 M by diluting part of the original solution (1-2 ml).

A hole the same diameter as the glass tube support is made with a cork borer on the opposite end of the chromatogram paper strip. The opening should be at such a distance from the lower edge that the strip nearly reaches the bottom of the lower vessel. After the strip is suspended, the solvent is carefully poured into the chamber through a small funnel inserted into a glass tube (diameter 12-15 mm) reaching the bottom of the chamber. The latter introduces an amount such that the level will be 1 cm below the line of acids placed on the paper. The upper vessel is placed upon the lower one,and along the line of their contact two turns of a wide strip of insulating tape is placed.

After 24 hours the chromatograms are removed from the chamber, suspended on a rack in a draft and the fan or room ventilator turned on for 15-20 minutes. They are left overnight in the room. On the next day the chromatograms are dried at 60-80° about 2 hours in order to eliminate the traces of formic acid. The suspended chromatograms are sprayed with the indicator through an atomizer and dried with a fan or ventilator; the position of the spots of the reference acids and the acid being studied are compared.

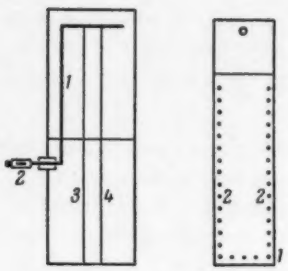

Fig. 1

Fig. 2

Fig. 1. Plan of the chromographic chamber.
1) Support; 2) Bunsen plug; 3 and 4) chromatograms.

Fig. 2. A chromatogram with marks for placing the acids and references on it.
1) Marks for acid placement; 2) references.

Partitioning Mixtures of Acids for Quantitative Determination

Standard leaves of chromatographic paper (52 × 64.5 cm) are cut into strips 13 cm wide and 64.5 cm long. A pencil line is drawn 2.5 cm from a narrow end of the strip; points are made 4 mm from one another on this line. With a very small capillary (or special pipette) a droplet of a 4-6% solution being studied is placed on the points in this line. After spreading, a droplet of solution placed on the paper should not have a diameter greater than 5 mm. For this reason it is essential to put on a very small droplet,which is easily done if the solution is not sucked into the capillary, which is only submerged and the excess fluid allowed to drain off; in this event there is enough solution in the capillary for innoculating 2-3 points. After the solution is placed along the entire line, the strip is dried; subsequently, 5-6 drops are placed on each point. In all 15-20 mg of acid is applied in this way. Two chromatogram strips are prepared by such a means. After the hole is made with the borer, both strips are suspended on the supporting rod at a distance of 2-3 cm from one another, the solvent is poured into the chamber and it is sealed with insulating tape.

The ambient temperature has a very considerable influence on the partitioning of a mixture of acids with an ether solvent. It should not exceed 20° while the chromatogram is in the chamber. The best results are obtained at a temperature of 12-14°.

The distance between zones of citric and malic acids on the chromatograms completed at 12-14° is 15-20 mm, at 20°, 5-10 mm and at 24-25° the boundary between citric and malic acid is indefinite.

The more widely distributed acids in plants, malic, citric, and tartaric, migrate along the paper appreciably slower than the solvent. This requires an extension of the chromatographing time up to 48-52 hours,and involves two procedures,since the sheet of paper is shorter than that traversed by the solvent in 48 hours.

After 24-26 hours the chromatogram is removed from the chamber (with the top of the latter immediately recovered to avoid escape of the ether vapor), suspended on a rack in a draft and turned by a fan or ventilator, (15-20 minutes is sufficient) and again placed in the chamber for 24-26 hours. The chromatogram is removed from the chamber after 48-52 hours with both methods, dried in the draft from a fan or ventilator, and left in the air until the following day. The chromatogram is dried at 60-80° for two or three days to eliminate the traces of formic acid, and the quantitative determination of acids is begun.

Determination of Acids

The chromatogram is laid on a sheet of clean paper, then the indicator (phenolphthalein) is gathered with a fine capillary,without sucking, and applied at a few points spaced at 10 mm along the long edge of the chromatogram (see Fig. 2). The points begin at the line where the acids were applied and end at the line to which the solvent ascended. In the zones where the acids occur, the line turns yellow, but remains azure between.

If the formic acid has not been completely lost, the stippled line becomes yellow over its entire extent; in this event it is necessary to continue the drying. The droplets are also placed on the opposite side of the chromatogram. At a 14 degree chromatographing temperature, the distance between zones occupied by individual acids in our experiments was: 35-40 mm between tartaric and citric, 15-20 mm between citric and malic and 70-75 mm between malic and succinic. Having determined with an orientational chromatogram the position of the acids, the boundary of each zone is made clearer for the exact determination of their stipple zones. Then, if the distance between zones is 0.5-3.0 cm, a line can be penciled from the middle of a blue stipple to a corresponding stipple on the other side. If the distance between zones of certain acids is 5-7 cm or more, the line is drawn 0.5-1.0 cm out from the edge of the zone to the blue stippled side. A few undelimited zones, corresponding to a set of acids, are obtained in the solution being studied. The chromatogram is cut up with scissors into strips along the pencil lines and the name of the acids marked down. A second chromatogram is then made. The corresponding zones of the two chromatograms are matched. The bands are cut up with scissors into short narrow ribbons, they are transferred to a small flask and extracted three times with water, adding 15 ml each time and heating to the boiling point. The solution is carefully poured into a 50 ml volumetric flask in order not to transfer the pieces of paper, chilled, brought to the mark with water, and shaken up.

Twenty-five ml of the solution of acids is transferred to a conical flask (100 ml), three drops of phenolphthalein added and the mixture is titrated with a 0.02 N alkali solution from a 5-10 ml microburette. On the end of the burette capillary is fitted a rubber stopper with a small tube containing the alkali concerned. The throat of the small flask for titration must be secured to the tube earlier.

Calculation of the di- and tricarboxylic acid content in the material being studied is made in this way: inasmuch as the ratio of acids in the solution being studied and that used for chromatographing is not altered, the amounts of alkali in the 0.02 N solution used to titrate the individual acids are added up, and the percent for each acid is calculated. The percentage for each acid is allotted on the basis of the percentage of the total weight of 0.1 N alkali used to neutralize the acids contained in the barium salts precipitated with 60% alcohol. The volume of 0.1 N alkali calculated for each one is multiplied by its corresponding coefficient in order to convert the alkali into milligrams of acid. Using 0.1 N alkali, the coefficient for tartaric acid is 7.5, malic 6.7, citric 6.4, succinic 5.9 and correspondingly calculated coefficients for other acids.

The values obtained for each acid are converted to the sample basis and also expressed in percent.

A sample calculation. 4.90 ml of 0.02 N alkali was used in neutralization of the tartaric acid extracted from a chromatogram and 25.75 ml was used on all the acids. Of this amount, tartaric used 22.53% In the neutralization of the acids from the barium salt, 108.8 ml of 0.1 N alkali was used; of this amount 22.53% was used on the tartaric, i.e. 24.5 ml, which corresponds to: $24.51 \times 7.5 = 0.184$ mg of acid.

In order to determine how complete was the extraction of the acids from the chromatogram, this experiment was set up: 1.0 ml of a solution containing tartaric, citric, malic, and succinic acids, each at a concentration of 0.2 N, was dropped onto chromatographic paper over an area of about one square decimeter, the paper dried with a fan and extracted with water. Another milliliter of solution was brought up to the volume of the extract. For neutralization of the acids, 58.80 ml of NaOH was used in the first case and 60.08 ml in the second. These results show that the acids are almost completely extracted from the chromatogram.

The exactness of determination of an acid in titration with 0.02 N alkali, as described above, is illustrated by results obtained from malic acid.

From a prepared, exactly 0.2 M solution of malic acid, aliquots were taken which, after suitable dilution, contained in 25 ml the amount intended for chromatographic determination of the acid:

Calc. mg/sample	Determined	Percent
6.70	6.49	96.4
6.70	6.50	97.0
3.35	3.20	95.5
3.35	3.27	97.9
1.675	1.69	100.9
1.675	1.675	100.0
0.838	0.858	102.8
0.838	0.864	103.1

Individual solutions of the acids, tartaric, citric, malic, and succinic, were prepared for chromatographic determination by the foregoing method. The amount of acid in each solution was determined by titration with 0.02 N alkali, after which a mixture of these acids was prepared from exact volumes of their solutions. A portion of this mixture (about 0.5 ml) was utilized for the partition and quantitative determination of the acids.

The results of the two experiments are shown in the table.

In using the method of paper chromatography for studying the organic acids of plants the following must be kept in mind: in the qualitative partioning of the acids on paper, a coincidence of the R_f values with the displacement of the acids being studied is no guarantee of their identity since there is room on the chromatogram for any of a vast number of acids. For example, when butyl alcohol is used as a solvent, the R_f values of malonic, oxycitric, and tricarballylic acids are close, nearly the same. Another group of acids, fumaric, aconitic, and itaconic, also have near R values.

It follows from the facts presented that in a number of cases, supplementary chemical characterization is required in order to determine the kinds of acids partitioned on a chromatogram. Frequently, specific color reactions are used but even they sometimes possess only relative specificity. For example, we found that the reactions between tartaric acid and resorcinol [9] and between gallic acid and ammonium molybdate, which are described as characteristic, are also obtained with certain acids, products of the first oxidation of sugars.

The method for partitioning a mixture of acids on paper described here gives amounts of them sufficient for determining the kind of acid by certain chemical methods: melting temperature, certain color reactions and determination of the molecular weight according to Rast [10]. A few milligrams of material is enough for these methods; on three to four strips of paper 13 cm wide, 20-30 mg of acids may be obtained from a mixture, which is unattainable with an ordinary qualitative separation.

Content of Acids in Mixture

	On chromatogram			% in the mixture after analysis of initial solution
	in ml 0.02 N alkali	in mg	% in mixture	
Tartaric 1	4.90	7.35	25.66	25.9
Tartaric 2	5.30	7.95	25.36	
Citric 1	7.67	9.82	34.24	34.6
Citric 2	8.46	10.83	34.55	
Malic 1	4.20	5.63	19.63	19.9
Malic 2	4.52	6.06	19.33	
Succinic 1	4.98	5.88	20.51	19.5
Succinic 2	5.51	6.50	20.74	

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DETERMINATION OF THE PHOTOSYNTHETIC PRODUCTIVITY OF PHYTOPLANKTON IN WATER USING C^{14}

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The process of creation of organic materials in water bodies because of photosynthesis by phytoplankton serves as a fundamental, and in the ocean is nearly the only source of organic substrate upon which all forms of life develop in the water. Therefore, it is very important to have methods which permit a rapid and very exact determination of the rate of primary production of organic materials due to phytoplankton photosynthesis in the entire water body.

However, up to the present, methods for determining photosynthetic productivity in a body of water have been inexact, were time consuming, and therefore did not satisfy the requirements of present day hydrobiology.

The problem of determining the total photosynthetic productivity of a body of water per square meter of water surface is substantially one of finding a method for the simultaneous determination of the magnitude of daily photosynthesis at various depths in the water body, from the surface to the depth where photosynthesis approaches zero. From these values, by one means or another, it is possible to easily calculate the daily photosynthetic productivity of the total water body. The magnitude of the daily photosynthetic productivity of the phytoplankton in various horizons of the water body is usually determined by the bottle method of Vinberg [1], the substance of which consists of the following: water samples taken from various depths in the water body are placed in illuminated and dark bottles on a rack in the water body at these same depths for a period of one day. After one day they are removed and the oxygen content of the bottles is determined by the Winkler method. There are serious deficiencies in this method. First of all, determination of photosynthesis on the basis of oxygen, at best expresses only the over-all balance of organic materials in the water, but not purely the photosynthetic productivity of the phytoplankton. Moreover, the sensitivity of the oxygen method has long been insufficient for many waters, especially marine and polar waters. For example, in order to determine the photosynthesis of a water stratum in the sea, Riley [2] incubated the bottle in the water for several days in order, in some degree, to detect photosynthesis which absolutely and completely distorts the true picture of the phenomenon. In the second place, this method requires prolonged stationing of ships and comparatively calm weather. This absolutely limits its use, especially for the study of large water bodies.

A correct representation of the primary productivity of water bodies can be obtained only by conducting a few-short-period series of determinations of the photosynthetic productivity at various points in the water body and during different seasons. This is dictated by the fact that organic materials in different parts of the water body and during different seasons are produced at unequal rates.

From what has been said, it follows that for a long time the necessity has been increasing for devising more convenient and exact methods for the determination of the photosynthetic productivity of phytoplankton which do not requre a day.

During a voyage on the "Galatee," Nielson [3] endeavored to work out such a method. He was able to select an empirical formula for calculating photosynthesis in various horizons of a water body from the values of photosynthesis in the surface layer, determined with C^{14}, by incubating the bottles on the deck of a ship with constant illumination. However, Nielson's formula appears valid only for the Indian Ocean.

While occupied with a study of phytoplankton photosynthesis in the Rybinskii water storage reservoir, we also encountered the need to devise a method permitting the determination of photosynthetic productivity without a day on station. Preliminary observations showed that it was impossible to calculate the amount of primary production of organic substances by measurement of photosynthesis at one point and another in the water storage reservoir as was done by Voronkov [4], since both the total phytoplankton biomass and photosynthesis vary greatly in different parts of the reservoir. In one of the surveys of photosynthesis in Rybinskii reservoir conducted for 7 days at the end of June 1955, we established that photosynthesis in different parts of the water body varied from 5 to 0.1 g C/m^2, i.e. ten-fold.

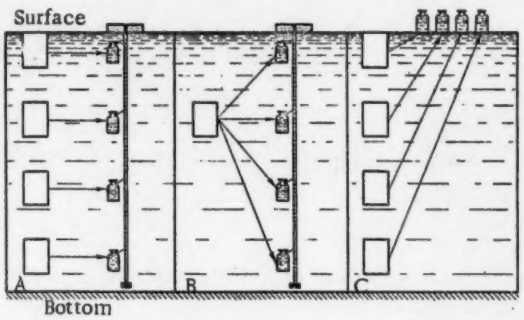

Fig. 1. Plan of experimental arrangement.

Thus, in order to obtain trustworthy results, it was necessary to work out a sufficiently exact and reliable method which would permit the determination of photosynthetic productivity in a water body during the voyage of a ship without spending a day on station.

Using radiocarbon, we were able to select a method which satisfies the indicated requirements. A description of this method and the results of its experimental testing are given below.

DESCRIPTION OF THE METHOD

Concretely, the problem of devising a new method for determination of photosynthetic productivity led both Nielson [3] and us, to see a way to assess photosynthesis in a water body, starting with the photosynthetic productivity of the surface layer of water, which could be determined by incubating a bottle not in the water body but in an aquarium on the deck of a ship. In order to do this, it would be necessary and sufficient to determine at each station the degree of influence on phytoplankton photosynthesis in a stratum of water exerted by the two fundamental factors which determine the character of the vertical distribution of the photosynthesis values: the penetration of light into the water for use by organisms on the one hand, and the unequal vertical distribution of the phytoplankton on the other hand.*

Numerous experiments conducted in the Rybinskii reservoir showed that the level of influence of these two factors on photosynthesis and also the value of photosynthesis itself in the surface layer can be easily determined by means of the isotope method.

1. Determination of Diurnal Photosynthesis in a Surface Sample of Water

A method for the determination of photosynthesis with the radioactive isotope of carbon, C^{14}, was devised by Nielson [3]. Kuznetsov [5] employed it for the study of photo- and chemosynthesis in lakes. Certain details were changed in our experiments and the determination made in the following way.

Water samples taken from the water body were immediately poured into transparent glass bottles with a volume of 250 cm^2. To them was added (with a syringe pipette) 1-2 ml of a solution of radioactive carbonate

*Without doubt, such factors as the distribution of parasites, pH, etc., also, to some extent, affect the vertical distribution of photosynthesis in a water body. However, their effect on photosynthesis can be considered together with the vertical distribution of the phytoplankton (see below).

($Na_2C^{14}O_3$) in 0.005 N KOH with a total radioactivity of $0.5\text{-}1 \cdot 10^5$ cpm/ml. The bottles were incubated for 0.5-1 day in an aquarium on the deck. At the end of the experiment, the phytoplankton in the bottles was fixed with alkaline formalin and filtered out on a diaphragm filter "pre-eminent" or No. 5. The filters with the phytoplankton precipitated on them are preserved in Petri dishes. In the laboratory at the conclusion of the expedition, the filters are treated with 1 N HCl in order to remove the radioactive carbonates and to determine in the phytoplankton the radioactivity of the organic matter which had been newly created in the process of photosynthesis (r).

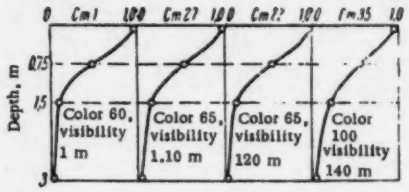

Fig. 2. Determination of the thickness of the photosynthesizing layer at various points in the Rybinskii reservoir. Photosynthesis survey July 27-August 3, 1955.

According to Nielson [3], in order to convert from the value of the radioactivity of organic carbon newly formed by photosynthesis (r) to the weight of carbon in milligrams, it is still necessary to determine the total radioactivity of the carbonate carbon CO_2 (R) found in the water after the addition of the solution of $Na_2C^{14}O_3$ to it, and the total amount of carbonate carbon + CO_2 (C_K) in the water. Samples of water are taken from the bottles into a test tube at the end of the experiment, before filtering, in order to determine total radioactivity (R). The radioactive carbonate and CO_2 in them are fixed by adding a few drops of 10% alkali free of carbonate. The test tubes are tightly closed with rubber stoppers and kept in an inverted condition until the end of the experiment. Analysis of the total radioactivity is carried out in the laboratory in the following way. About 3 ml of 0.1 N KOH (free of carbonates) is poured into a test tube. Then to it is added: about 1 ml of solution containing 0.3% $Na_2C^{14}O_3$ and 10% NH_4Cl, about 2 ml of the liquid being analyzed and about 2 ml of a 1 M $BaCl_2$ solution. The test tube is heated 10 minutes on a water bath at 80° after which the $BaCO_3$ precipitate is filtered out through a No. 1 and No. 2 diaphragm filter. The precipitate is washed from the walls of the tube and filter funnel with a hot solution of 1% $BaCl_2$ + 5% NH_4Cl in 0.1 N KOH. After filtration, the filtrate is dried and weighed exactly to the fourth place in order to determine the weight of $BaCO_3$ for calculating the internal absorption of radiation in the mass of a precipitate. The radioactivity of the $BaCO_2$ precipitate is determined on the dried precipitate immediately after filtration, in view of the fact that radioactive barium carbonate exchanges its carbon with the carbon of the CO_2 of the air, and over a period of time the radioactivity of the precipitate on the filter decreases.

In order to avoid introducing corrections into the calculation, the $BaCO_3$ was filtered through a funnel of such a diameter that both the funnel for filtering the radioactive phytoplankton and the filter with the $BaCO_3$ precipitate are miscounted by the counter under the same circumstances as the filter with the phytoplankton.

To find the correction for internal absorption, we used the graph relating internal absorption to weight of the $BaCO_3$ precipitate shown in the paper by Nielson [6].

We determined the total content of carbonate carbon + CO_2 by two methods: direct titration in water, and titration after distillation in alkali. The first analysis was performed on shipboard according to the following scheme. A 100 ml water sample was poured into a volumetric flask and 0.1 N KOH added to a weak pink phenylphthalein color. At this point all forms of carbonic acid are converted to bicarbonate. Then the bicarbonate is titrated with 0.5 N HCl in the presence of the mixed indicator methyl orange + methyl blue to a pink color. The direct titration method is not completely exact since humic acids and other materials soluble in water are titrated together with the carbonate.

The method of titrating CO_2 after distilling it in alkali is more exact. Analysis by this method is conducted in the laboratory at the end of the expedition. The water sample is fixed for analysis by adding 2-3 ml of 0.1 N KOH. In a special apparatus the carbon dioxide is expelled from the carbonates with acid, and distilled with a stream of air into 0.1 N alkali, after which it is titrated with HCl in the presence of $BaCl_2$ [7].

The amount of organic carbon dioxide newly formed in photosynthesis (C_{FP}) was calculated from the phytoplankton radioactivity (r), total radioactivity of the carbonates + CO_2 (R) and their total concentration in water (C_K) according to Nielson's formula [3]:

$$C_{FP} = \frac{C_K \times r}{R}.$$

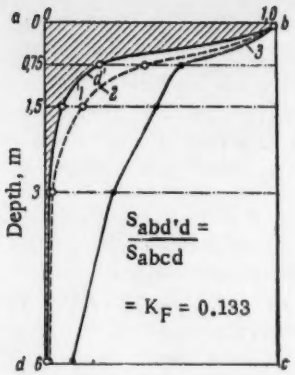

Fig. 3. Diagram for determination of coefficient K_F. Volga tributary, August 12, 1955. 1) Corrected summary curve of the coefficient $K_c = K_m \times K_p$; 2) curve of coefficient K_T; 3) curve of coefficient K_p.

Parallel treatments in dark bottles were set up as controls for the absorption of radioactive carbonate carbon not connected with photosynthesis (chemosynthesis and dark fixation). As the observations showed, it was not necessary to set up a control experiment every time since C^{14} absorption by algae in darkness does not exceed 0.5% of C^{14} absorption by photosynthesis in a lighted bottle.

The sensitivity of the determination of photosynthesis by the isotope method on the average is of the order of magnitude of $1 \cdot 10^{-4}$ mg C/l. Therefore, with the aid of C^{14} the photosynthesis of a known number of algae cells can be determined.

2. Determination of the Dependence of Photosynthesis on the Light Transparency of Water and Finding the Correction Coefficients (K).

The dependence of photosynthesis on light transparency is determined in twelve-hour periods in the following way. A sample of water is taken at some average horizon (Plan B, Fig. 1). The water is distributed in equal amounts into transparent bottles and to them is added an exact volume of radioactive carbonate solution, with a total activity of $1\text{-}5 \cdot 10^6$ cpm/l. Then the bottles are placed on a rack in different horizons of the water body from the surface to the horizon with zero photosynthesis.* The experiment was set up from 12 noon to evening or from evening to 12 noon, in order that the algae might use the whole gamut of change in illumination in the water body, from the diurnal maximum to the diurnal minimum.** At the end of the experiment, the bottles are removed from the water body, the algae in them fixed and immediately filtered out on a diaphragm filter.

It is evident that with a such an experimental apparatus, the values of photosynthesis in bottles incubated in different horizons will express the dependence of photosynthesis in these horizons upon the illumination of the water body since the unique factor here which affects photosynthesis is the decrease in illumination with depth. All of the other factors, which in some way affect photosynthesis are equal in all bottles. On the other hand, however, the total content of carbonates + CO_2 (C_K) and total radioactivity (R) are also equal in all bottles. Therefore, the magnitude of photosynthesis in the bottles will be directly proportional to the radioactivity of the corresponding filters. From what has been said, it follows that the radioactivity of the filters containing the phytoplankton which were incubated in different horizons, will reflect the dependence of photosynthesis upon the light transparency of these horizons.

Calculation of the ratios of the radioactivities of the filters from the bottles incubated at different depths to the radioactivity of the filter incubated at the surface with maximum light also tells us the degree of decrease in photosynthesis in the water body in relation to the decrease of light with depth. We have designated this ratio as coefficient K, which corrects for the dependence of photosynthesis in the water body upon the light transparency of the water. The value of coefficients K_T varies from one for the surface layer of water to zero for all those horizons to which light does not penetrate. If the value of the surface photosynthesis is multiplied by the coefficient K_T, found for separate horizons, we obtain the values of photosynthesis which we would observe in these horizons if the body of water were completely saturated with phytoplankton and parasites. By graphing the values of the coefficient K_T, we obtain a graphical expression for the dependence of photosynthesis upon illumination of the water body. This curve provides a graphic depiction of the thickness of the photosynthesizing layer of the water body (Figs. 2, 3). The investigation we have presented showed that the curve for the dependence of phytoplankton photosynthesis upon illumination does not coincide with the curves

*In view of the great sensitivity of the isotope method this experiment is best set up in the following way: the bottles containing the water are attached to the rack and placed in a black sack. Then the isotope solution is added to all bottles, they are submerged in the water body and the sacks are removed from them in the water.

**Diurnal experiments give more reliable results since phytoplankton begin to multiply and this distorts the true picture.

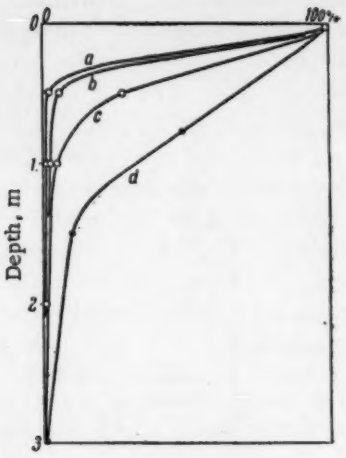

Fig. 4. Variation of illumination in a water body in relation to fluctuations in the value of the surface illumination.
Volga tributary, July 12, 1955.
Illumination (% of surface):
a) at 7 P.M.; b) at 1:20 P.M. (sun behind clouds); c) 1 P.M. (clear); d) thickness of photosynthesizing layer.

of change in illumination with depth, which were measured with a photometer. Figure 4 gives data on the determination of illumination in a water body at various depths at different times of the day and under different atmospheric conditions. These determinations were made during one day and at the same station. At this same station the dependence of photosynthesis on illumination (coefficient K_T) was determined. The results of the determinations are also shown on this graph. As can be seen from the data presented, the illumination in a water body decreased more rapidly with depth than does photosynthesis, even with maximum illumination in the surface layer of water. Apparently, the cause for this is that the coefficient for use of light by algae for photosynthesis is not the same at different depths, but smaller in the surface layers and larger with depth. Thus, the magnitude of the coefficient K_T cannot be measured with a photometer.

Determination of the dependence of photosynthesis on light in the Rybinskii reservoir at different times during the vegetative period showed that it is nearly uniform through the whole water body during certain intervals of time (Fig. 2). Changes in this dependence are linked basically to shifts in the type of phytoplankton and with fluctuations in the color of the water, which occur quite slowly. Therefore, it is not essential to measure the coefficient K_T at every station. In the Rybinskii reservoir, for example, it was sufficient to determine the value of K_T at 2-3 stations once or twice per month.

3. Determination of the Dependence of Photosynthesis in a Water Body Upon the Vertical Distribution of Phytoplankton (Finding the corrected coefficient K_P)

As pointed out above, in order to calculate the photosynthetic productivity of a water body from the magnitude of photosynthesis in the surface layer of water, it is necessary to evaluate in addition the illumination factor and also the vertical distribution of the phytoplankton. The method of direct quantitative calculation of the phytoplankton a priori would appear to be irrelevant to the analysis of the vertical distribution of phytoplankton. The reason for this does not consist only in the fact that a direct method of phytoplankton determination is exceedingly laborious and quite subjective. The main reason lies in the absence of a proportionality between the total biomass of cells determined by a direct count and ability to photosynthesize, which implies a different physiological state of the cells under differing functional conditions, and also implies that, in addition to the living cells, dead and dying phytoplankton cells are included in the direct calculation.

In order to study the possibility of calculating the effect of the vertical distribution of phytoplankton upon photosynthetic productivity, it was necessary to find a method which permitted the determination of the distribution of vitally active phytoplankton able to photosynthesize. The isotope method we worked out, which permitted an easy and rapid determination of the vertical distribution of vitally active phytoplankton in a water body, served this requirement. The experiment was conducted according to plan B (Fig. 1). The water samples were taken from the same horizon in which the coefficient K_T was determined. The water was poured into transparent glass bottles. Immediately, exact volumes of $Na_2C^{14}O_3$ solution calculated to have $0.4\text{-}1.5 \cdot 10^6$ cpm/l (depending on the amount of phytoplankton in the water) was added to the bottles. The bottles were incubated in an aquarium on the deck for 1-2 hours under uniform illumination conditions after which the algae in the bottles were fixed. The radioactivity of filters in such an experiment will be proportional to the photosynthesis in the bottles.

In turn, photosynthesis depends upon the original number of vitally active cells of phytoplankton in the respective horizons, since under the experimental conditions just this factor limits photosynthesis. On the other hand, the time required for the experiment guarantees distortions in the original picture of phytoplankton distribution, which may occur as a consequence of multiplication of the phytoplankton in the bottle during the

experiment. The magnitude of photosynthesis (and consequently of the value of radioactivity of the filters) in these experiments expresses not only the influence of the vertical distribution of the phytoplankton upon photosynthesis, but also the influence of the concrete environmental factors which accelerate or retard this process (pH, parasites, etc.).

It is evident that the ratio of the radioactivity of filters obtained from samples at different depths to the radioactivity of a filter from the surface sample will correlate with the effect of the vertical distribution of the photosynthesizing phytoplankton upon the photosynthetic productivity in the water body. We signify this ratio as the corrective coefficient K_p. In multiplying the value of the surface photosynthesis by the coefficient K_p found for individual horizons, we obtain the value for photosynthesis which would be observed in the respective horizon in case all the layers of the water body were equally illuminated.

In the event of a variable content of carbonates + CO_2 in different horizons, the direct proportionality between the magnitude of photosynthesis and the radioactivity of the filters will be upset, and for its reestablishment it is necessary to determine C_K on the different horizons. However, the latter is essential only for determining the vertical distribution of the phytoplankton, and is not linked with the determination of its productivity. In order to find the coefficient K_p the distribution of the values of C_K need not be calculated, since correction of the distribution of C_K by horizons automatically occurs in the corrective coefficient K_p.

4. Calculation of Photosynthetic Productivity Beneath 1 m^2 of a Water Body Surface.

In order to find the over-all corrective coefficient indicating the degree of common influence of the illumination factor and the factor of vertical distribution of phytoplankton upon photosynthesis in a water body, it is sufficient to cross-multiply K_p and K_T. The correction coefficients obtained by cross-multiplying coefficients K_p and K_T for each horizon are designated as summary coefficients K_C. These coefficients also show how the magnitude of photosynthesis varies with depth in the respective horizons. Therefore, by multiplying the value of diurnal photosynthesis in the surface sample (C_{FP}) with the coefficients K_C we can calculate the magnitude of photosynthesis in these horizons, which in the final analysis makes it possible to determine the over-all photosynthetic productivity in a water body. In practice the determination of photosynthetic productivity is made in the following way: with a bathometer, water samples are taken from the ship at various depths from the surface to the depth where there is no photosynthesis. The horizons from which the samples are taken were selected in relation to the thickness of the photosynthesizing layer in the water body being studied. In general, samples from 4-5 horizons, including the surface, are sufficient for determination of the photosynthetic productivity. In the bottles containing surface water the magnitude of diurnal photosynthesis is determined by incubating the bottles with the added isotope in an aquarium on the deck. For this it is better to select a half-day incubation period, since the isotope method gives more reliable results at compartively short exposure [8]. In the latter case all samples obtained before noon are held from noon to darkness and all samples obtained after noon are held from evening to noon the following day. A simultaneous series of bottles containing water samples from all horizons is incubated, with the isotope present for 1-2 hours, on the deck, in order to determine the coefficients K_p. The correction coefficients K_T are determined in general several times per month, along with the shift in type of phytoplankton and the fluctuation in water color.

The filters containing the radioactive algae are preserved in Petri dishes on filter paper moistened with a few drops of formalin, until the end of the expedition. The material is treated after the expedition is completed. In order to find the summary coefficient K_C, the values for the radioactivity of the filters containing the algae obtained for each station are listed in a calculation table (see Table). After the value of K_C is found, a curve (See Fig. 3) is constructed and on the basis of the ratio of the area bounded by the curve to the total area of the graph, the final correction coefficient K_p is found. This coefficient indicates what part the photosynthetic productivity actually observed in a water body is of that which would occur if there were a uniform distribution of light, phytoplankton and other factors in the water body. The total photosynthetic productivity of the water body (C_F) is calculated from the value of the surface photosynthesis (C_{FP}), depth to which measurements were made (1), and the value of the coefficient K_F according to the formula $C_F = C_{FP} \cdot 1 \cdot K_F \cdot 1000$ g C/cm^2.

It ought to be noted that in a majority of cases there is no necessity to construct a curve in order to find the correction coefficient K_F for each station. It is quite sufficient to construct one general curve for all stations

TABLE

Comparison of Two Methods for Determining Diurnal Photosynthesis in a Water Body

Rybinskii Reservoir in Koprino Village. Total depth 12 m July 19, 1955. Color blue-green. Content of carbonate carbon + CO_2 carbon in water (C_K) 13.8 C/l. Total radioactivity after isotope addition C^{14} (R) – 290,000 cpm/l

Immediate determination by Plan A (Fig. 1)				Determination by calculation from the value of surface photosynthesis with a correction coefficient K_C introduced					
				determination of coefficient K_T corrected for light (plan B, Fig. 1)		determination of coefficient K_p corrected for phytoplankton distribution		overall correction coefficient K_c ($K_p \times K$) ($K_p \times K$)	diurnal photosynthesis, mg/l
depth, m	radioactivity of algae on filter at end of experiment cpm/100 ml (after dark bottle deducted)	diurnal photosynthesis mg C/l	ratio of photosynthesis at a depth to that at surface, as unity	radioactivity of algae on filter cpm/100 ml (after dark bottle deducted)	coefficient K_T	radioactivity of filter cpm/100 ml (after dark bottle deducted)	coefficient K_p		
0	4222	2.02	1.00	1147	1.00	885	1.00	1.00	2.02
0.75	622	0.296	0.148	652	0.57	210	0.237	0.135	0.274
1.5	137	0.064	0.032	327	0.285	106	0.106	0.03	0.060
3.0	4	0.002	0.001	43	0.03	66	0.07	0.002	0.004
6.0	3	0.00015	0.0007	5	0.004	31	0.03	0.001	0.0002

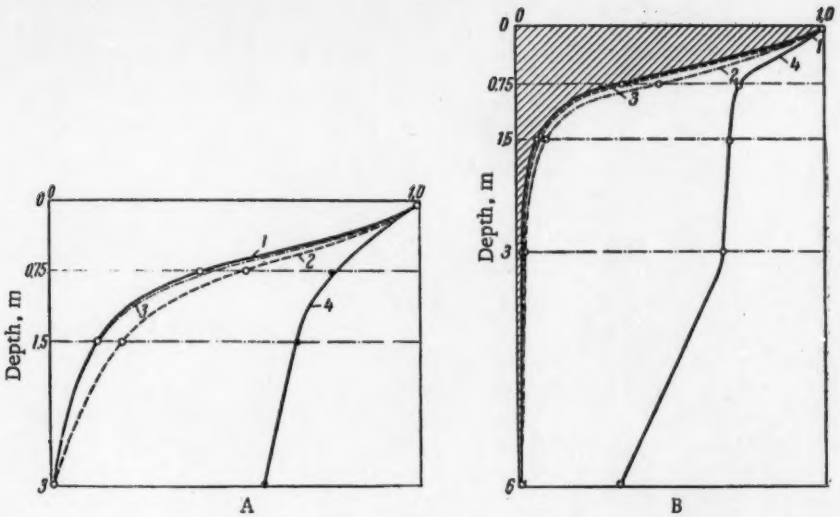

Fig. 5. Comparison of two methods for determination of photosynthesis. Volga Tributary, A) Determination July 22, 1955; B) determination August 15, 1955; 1) coefficient K_F; 2) coefficient K_T; 3) photosynthesis determined by plan A (Fig. 1); 4) coefficient K_p.

occupied for 0.5-1 day. In order to construct a general curve, it is necessary to take the average value of the coefficients Kp observed after a day on different stations and cross-multiply them with the corresponding values of coefficient K_T, and then graph the values of K_C obtained. It is not difficult to see that by a similar method of calculation, we can sum up the influence of the vertical distribution of phytoplankton upon diurnal changes in productivity of photosynthesis. In this way can be avoided one of the chief deficiences of the old bottle method which, of course, consists of the impossibility of measuring the diurnal changes in vertical distribution of phytoplankton. In conclusion we point out that by using the new method on the Rybinskii reservoir, during a day we sampled up to 15 stations for determinations of photosynthetic productivity while the ship traveled in excess of 100 km.

In order to experimentally test the described method, we conducted a number of experiments in a water body in which we compared the values of the photosynthetic productivity of the phytoplankton in separate horizons, calculated with the help of the correction coefficients K_C, from the values of the surface photosynthesis, with the values of photosynthesis which we obtained by a direct determination in these horizons according to plan A (Fig. 1). The experiments lasted 0.5 day.

The graphed experimental results (Fig. 5) show that the calculated values of photosynthesis and those obtained by direct determination in a twelve hour experiment on different horizons correspond quite closely. In this way the determination of photosynthetic productivity in a water body, with the aid of correction coefficients, is quite reliable. More than that, in the event the diurnal change in the vertical distribution of phytoplankton is calculated, which can be studied by finding the average daily value of coefficient K_p, the method of calculation will be more reliable than the method of direct determination on a day-long station according to plan A (Fig. 1) since, in the determination according to plan A, the phytoplankton distribution which existed at the time the experiment began is fixed.

SUMMARY

A method has been developed for determination of the productivity of phytoplankton photosynthesis in water. Radioactive carbon C^{14} is employed and no daily stations are required. The productivity of photosynthesis per square meter of water surface body is computed from the magnitude of photosynthesis in a surface layer of water, and accounts for factors influencing the distribution throughout the depth of the water. An experimental check of the new method showed it to be suitable as a means of studying the primary productivity of water bodies.

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REVIEW

N. A. Maksimov. A Short Course in Plant Physiology, Ninth Revised Edition, Edited by Adademician A. L. Kursanov assisted by Prof. I. I. Gunar, L. V. Mozhaev, A. A. Nichiporovich, V. I. Razumov, E. I. Ratner, F. D. Skazkin and S. V. Tageev. Sel'khozgiz (1958)

During the time that our national plant physiology has existed as a science and as a subject taught in the higher schools, the universities and agricultural institutes, no single book has been published so many times as "A Short Course in Plant Physiology" by N. A. Maksimov.

Such an interest in this text book, undiminished for thirty years, is explained by the great scientific authority of the author and by his pedagogical skill as a teacher in the higher schools.

Skill in selecting from abundant factual material the most valuable, which he puts into perspective for future development, the objective, critical evaluation of theoretical hypotheses, the organic matching of scientifically significant material and its agricultural value; all these things contributed to the success of the textbook of N. A. Maksimov. The significance and literary merits of the course in plant physiology are the over-all clarity andsimplicity in discussion of complex problems and the great internal logic and orderliness.

The eighth edition of the textbook appeared in 1948, late in the life of the author. During the past ten years a number of sections on the physiology of plants were supplemented by new facts, frequently of basic significance and even also on the basis of their theoretical meaning. Much, especially, has been done in the realm of the physiology and biochemistry of metabolism as a result of a wide usage in physiological experiments of a number of new, exceedingly effective methods of investigation, radioactive isotopes, chromatography, optics, etc.

It would require great and responsible effort for any scientific group to undertake the task of preparing the ninth edition of "A Short Course in Plant Physiology." It is not easy to select from the tremendous amount of contemporary scientific factual material the more valuable, which merits study and which facilitates the further development of the science. In selecting, it was necessary to consider also the size of the book, and at the same time not disrupt its basic character, literary merit, its brevity, simplicity, and lucid language.

In our opinion the people who participated in the preparation of the ninth revised edition of the textbook fully succeeded in their task. It is true that the course in physiology has not been supplemented in all sections with an appreciable number of exact facts and ideas which science has obtained during the last ten years.

The sections dealing with aerobic nutrition, plant respiration, and the conversion and translocation of material are especially complete. The volume and character of the additions in these sections objectively reflect the facts of the situation, since no single section of plant physiology has developed as intensively as that dealing with metabolism.

The rest of the sections of the course contain fewer additions, but even in them a number of substantial facts and theoretical presentations which have appeared during the last ten years are expressed.

The section "Carbon Assimilation" has been appreciably expanded chiefly because of new factual data on the intimate chemistry of photosynthesis obtained through the use of new methods of investigation, the use of radioactive carbon in conjunction with chromatography and ion exchange resins. An entire paragraph has been inserted concerning tagged-atom techniques and certain other of the very newest methods for investigation of photosynthesis and other aspects of metabolism. Great attention has been given here to Tsvet's method of chromatography, to which is due the beginning of contemporary methods of adsorption and partition chromatography.

Calvin's photosynthetic cycle has been presented as the last achievement in the study of the chemistry of photosynthesis. It seems to us it would be worthwhile here to introduce this cycle not in a pictorial form but in the form of chemical formulae, since this has been done by Calvin himself. Chemical formulae in a textbook give a more lucid and graphic presentation of the sequences and chemistry of the reactions than does a figure with the names of the substances. It would be worthwhile here to mention two new enzymes, transketolase and transaldolase, whose role also falls within the limits of photosynthesis.

In this section an old table of the changes in the spectrum of chlorophyll absorption with increasing chlorophyll concentration in solution has been successfully replaced by Zscheile's up-to-date tables which give the quantitative spectral characteristics of absorption by living leaves and the action spectrum of photosynthesis of wheat leaves. Illustrative material on the uses of artificial light sources in the thermal economy has been inserted,and also some concerning the relation between yield and the intensity of photosynthesis. The section dealing with plant respiration is also enriched with additions in accordance with new facts and theoretical concepts. Not only items concerning the over-all biological role of respiration as a source of energy have been expanded but also as a process which initially produces intermediary substances used in the synthesis of diverse compounds, primarily proteins. New paragraphs have been inserted on "Respiration and oxidation-reduction reactions," "Contemporary concepts of the chemistry of respiration," "Respiration as a source of energy," "Enzymes of the respiratory cycle," and "Respiration and the vital activity of plants."

The insertion of the new paragraphs,it must be admitted, is exceedingly valuable,since understanding a number of the facets not only of the chemistry of respiration but also its biological role,would be perplexing during the perusal not only of the special literature on plant metabolism,but also of the contemporary popular scientific literature.

Here it would be worthwhile to note that in giving great attention to the question of respiration chemistry, the author for some reason or other did not mention additions, if only for general emphasis, to contemporary findings and concepts concerning direct oxidative respiration and enzymes,which are even more pertinent since certain of them participate in photosynthesis.

In paragraph 63 in the part concerning methods of respiration, the figures and reference to the endiometer and apparatus of Pettinkofer could be eliminated without any loss;since the latter has not been used for nearly half a century now and the endiometer for even longer. A contemporary teacher who is not a specialist could get the impression that this is a modern method of investigation. In general, the textbook has been changed by new figures in a whole series of cases which would probably have been done by the author of the course himself. Space ought to be taken here to note that in a number of cases the description of methods of investigation should have been confined to only a brief presentation of the principles of the methods instead of describing them,since it is completely impossible in a general course to present all the details.

The cyclic formulae of the important sugars in the plant world, glucose, and fructose in the pyranose and furanose form, and also the structural formula of sucrose have been presented in the section dealing with conversion and translocation of substances in plants.

This section is appreciably more detailed than the preceeding edition of the course which described the structure of starch and presented a scheme for its construction. The most important amino acids of the aromatic and hetero-cyclic series are given in structural formulae and an equation is given for formation of peptides from amino acids. New data are given on the synthesis of amino acids in root systems from ammonia and keto acids and the translocation of amino acids in the aerial organs; attention is devoted to nitrate reduction in plants. Information on the nature of enzymes and their effect on the foundations of modern research is appreciably expanded. Additions here concern the chemical constitution of enzymes, their structure, and classification and also the problem of their reversibility of action. Data have been introduced concerning phosphorylase, and transfer enzymes, with the aid of which reverse enzyme reactions are feasible. The paragraph on movement of organic materials in plants has been supplemented with new facts which show that translocation is not a passive process, but occurs as a coordinated physiological event closely linked to tissue respiration and metabolism. It seems to us that Myunkh's theory of the mechanism of translocation in plants could be excluded from the new edition without any loss.

In addition substantial changes have been made in supplementing the section on " Organization of plant cells and their behavior." Part of the paragraph in the eighth edition "General concepts concerning the colloidal

state and its significance for life phenomena" has been omitted and the other part transferred to the paragraph on the physiological features of protoplasm. There are additions on cell structure, proteins, and the molecular weight of certain of them. New tables are given for analyses of protoplasm, comparative viscosity of cell sap, the cytoplasm of plant cells, and certain organic materials.

The concepts of oxidation-reduction potential introduced in the paragraph are not lucidly formulated, are not in the style of the textbook, and can only be referred to detrimentally.

Of fundamental significance is the replacement in the fourth to eighth edition of the course of the title of the paragraph "Plasma permeability and the phenomenon of plasmolysis" by "Selective permeability of protoplasm." Unfortunately the more recent views of Nasonov and Troshin on the nature of permeability are not presented in the paragraph on permeability.

The concepts of free and bound water are used in the section on plant water regime but determination of them is not discussed. The question of water entry into the root system for some reason does not treat Sabinin's recent point of view and cites earlier views. In this section new facts are introduced concerning the wilting coefficient, the effect of soil temperature on water uptake and the biochemical processes in roots, and certain others. Ditmer's data on the extent of the root system is greatly overemphasized since they were obtained under artifical conditions and it would be worthwhile to substitute Rassel's results, which were obtained under natural conditions.

Certain additions have been made to the section "Use of nitrogen and mineral substances by the plant;" how plants use organic phosphorous compounds, the phosphobacteria, and the role of soil microorganisms and their secretions in plant nutrition. The old experiment of Osterhout on ion antagonism which has been presented could be replaced by the new more exact experiments of Lubbert. Little has been added on the problem of the role of sulfur and potassium in plants, in particular concerning the significance of the thio-ethers involved in metabolism and the role of potassium in synthesis processes.

The section "Plant growth and movement" contains an appreciable number of additions, some of which have fundamental significance; among them attention has been given to the causes of cell growth during the elongation stage, not only because of an increased water influx but also because of an increase in the amount of protoplasm synthesized from the fluid material being imported, as is indicated by the increase during this stage of a whole series of indicators (all forms of nitrogen, phosphorous, sugars, etc.). An important addition is the indication that the role of auxin is appreciably greater than ascribed to it until now; auxin is not only a factor in cell elongation, but also affects their division; for example, it can activate growth of the cambium. The prevalence of the aerobic type of cell respiration during the second stage of growth is noted. There are also a number of additions on other aspects of growth, for example, the influence of light on growth, chemotropism, etc. New facts are presented concerning the stimulatory action of certain vitamins upon plant growth in sterile culture on mineral media, and information has been given on new herbicides.

In the section dealing with the physiology of plant development in the new edition of the course, additions are made which remove somewhat the dogmatism and categorism of a number of positions which were held at the time the eighth edition was issued. An addition has been made which excludes the quite categorical formulation of growth versus development of plants; the reciprocity of growth and development is closely stressed. On the basis of new facts, the opinion is expressed that the treatment of phasic changes and their irreversibility until recently was too one-sided. Appreciably greater space has been devoted in the section to photoperiodism; new facts have been presented which point out the importance of the effect of stimularoty substances in changing the photoperiodic reaction in certain plants. Some condensations of the author's text, which make formulation more explicit, have been made in this section.

For some reason, no additions, even though very brief, are made from the new facts concerning gibberellin, either here or in the section on growth.

The section "Plant resistance to unfavorable conditions" contains a number of additions which expand the information on this problem; for example, hardening of woody plants to cold is accomplished in two steps: the first stage of hardening occurs at a temperature not much above zero, the second at lower subzero temperatures. Aspects of the close link between frost resistance and the growth processes are somewhat restricted. The curtailing of growth has not always been linked to an increase of frost and cold resistance. During the

annual cycle of overwintering plants, two sharply distinct physiological conditions are noted, the vegetative and the winter hardy which are characterized by their metabolic tempo.

In the paragraph about xeromorphism and drought resistance of plants, new facts are presented concerning the dissimilar sensitivity of plants to drought during different periods of their development, and the effect of soil water deficiency on metabolism and on the processes of development of the reproductive organs. For better understanding, the classification of the xerophytes ought to be given in a more developed form or not presented at all.

It should be noted that in a majority of cases the additions are successfully meshed with the basic text of the author, in their character and style of presentation.

All that has been said provides a full basis for welcoming the timely appearance of the ninth, supplemented edition of "A Short Course in Plant Physiology" by N. A. Maksimov.

For a number of years this course has been not only a textbook for agricultural teaching institutions, but also a valuable educational assistant for the university biological faculties. A new edition of the course, appreciably supplemented, which is presented in agreement with contemporary attainments of plant physiology, will still serve for a number of years as a valuable work for the study of the basis of plant physiology.

It is disappointing that many misprints crept into this good textbook, part of them not having been noted in the galley.

This report is a result of the consideration of "A Short Course in Plant Physiology" by associates of the faculty of plant physiology of Leningrad State University consisting of Prof. S. V. Soldatenkov, O. A. Grechukhin, A. N. Panteleev, A. N. Kubl, T. M. Bushuev, and V. V. Pinevich.

S. V. Soldatenkov

ERRATA

Volume 5, Number 4, the Fig. 1 on page 375.

A shift of the number scale occurred which distorted the meaning of the chromatogram. The figure should read:

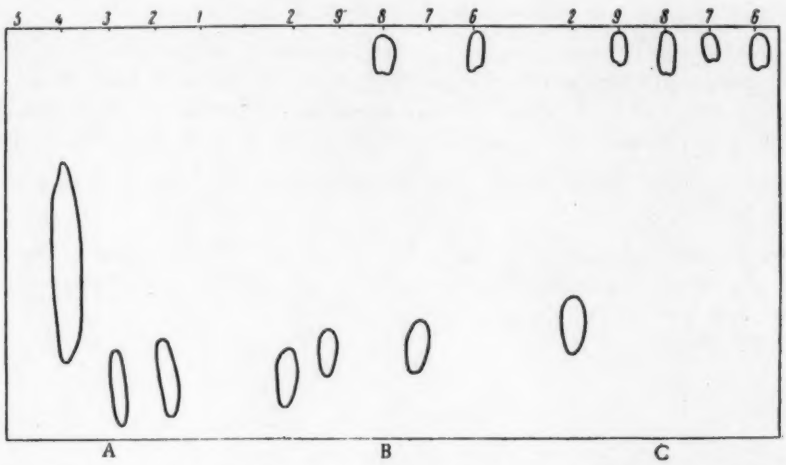

Fig. 1. Chromatograms of wheat coleoptiles treated with stimulating and inhibiting doses of potassium salts. A) stimulating doses of 2,4,5-T and TAB for 24 hr; B) stimulating doses of 1-TG and d-TG for 24 hr; C) inhibiting doses of 1-TG and d-TG for 48 hr. Spots of the following were placed on the starting line: 2) 2,4,5-T; 4) TAB; 6) 1-TG; 8) d-TG, as well as the substances separated from solutions of the compounds with which the wheat coleoptiles were treated: 1) water; 3,5,7 and 9) potassium salts; 3) 2,4,5-T; 5) TAB; 7) 1-TG; 9) d-TG.

Volume 5, No. 6 the Table on page 491 should read:

Components	Concentrations M	Components	Concentrations M
$MgSO_4$	3×10^{-3}	$Fe_2(SO_4)_3$	6×10^{-6}
$Ca(NO_3)_2$	1.2×10^{-3}	$MnSO_4$	3.7×10^{-6}
Na_2SO_4	1.4×10^{-3}	$ZuSO_4$	9.4×10^{-6}
KNO_3	8×10^{-4}	H_3BO_3	1.2×10^{-6}
KCl	9×10^{-4}	KI	4.5×10^{-7}
$NaH_2PO_4\cdot H_2O$	9×10^{-5}	Sucrose	6×10^{-3}

Volume 5, Number 6

Page:	Line:	Read:	Should read:
496	31	4a, b and c	4d, e
498	34	Buirmzer	Biurzmer
506	34	20 mg P_2O_5	200 mg P_2O_5

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci.-Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiia	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiia	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Embriol.	Arkhiv Anatomii Gistologii i Embriologii	Archives of Anatomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiia	Biokhimiia	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiia Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Biull. Eksptl. Biol. i Med.	Biulleten Eksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Biull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Biulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Eksptl. Khirurg.	Eksperimentalnaia Khirurgiia	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiia i Toksikologiia	Pharmacology and Toxicology
Farmatsiia	Farmatsiia	Pharmacy
Fiziol. Rastenii	Fiziologiia Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenova Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariia	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiia Akademii Nauk SSSR, Seriia Biologicheskaia	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiia Tikhookeanskogo N. I. Instituta Rybnogo Khoziaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiia	Khirurgiia	Surgery
Klin. Med.	Klinicheskaia Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo(po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaia Parazitologiia i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaia Radiologiia	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiia	Mikrobiologiia	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhiat. i Psikhogig.	Nevropatologiia, Psikhiatriia i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediia, Travmatologiia i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriia	Pediatriia	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Endokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniia Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaia Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journals
Stomatologiia	Stomatologiia	Stomatology
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriia	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics

* LIBRARY OF CONGRESS TRANSLITERATION SYSTEM.

(continued)

(continued)

Abbreviation	Journal	Translation
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiia	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiia	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiia, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoiuz. Gidrobiol. Obshchestva	Trudy Vsesoiuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoiuz. Inst. Eksptl. Med.	Trudy Vsesoiuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiia	Urologiia	Urology
Uspekhi Biokhimiia	Uspekhi Biokhimiia	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiia	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriia Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universiteta, Seriia Biologii i Pochvovedeniia	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiia	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariia	Veterinariia	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniia	Voprosy Pitaniia	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaia Laboratoriia	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiia	Journal of General Biology
Zhur. Vysshei Nerv. Deiatel.	Zhurnal Vysshei Nervnoi Deiatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

A.I.B.S. Russian Monograph Translations

The AIBS is in the process of expanding its Russian Translations Program extensively. Funds to subsidize translation and publication of important Russian literature in biology have been obtained from the National Science Foundation, as part of a larger program to encourage the exchange of scientific information between the two countries. The following monographs have been published:

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